HONI SOIT QUI MAL Y PENSE
DIEU ET MON DROIT

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Sodium chloride	0.5
Calcium carbonate	0.5

To this grain mixture 5% of butter was added after grinding. Fresh whole milk was fed daily and lettuce once each week.

EXPERIMENTAL RESULTS

When the pituitaries from group I were assayed against those of group II the glands from group I yielded slightly more lactogen than those from group II. On the average, the pituitary glands in group I contained 10.84 bird units per gland, those in group II assayed 9.46 bird units. This slight difference in the lactogen content of the pituitary glands in these two groups was due primarily to a difference in the concentration of the hormone in the glands, because in group I the glands contained 1.2 bird units per mg. of pituitary tissue and those in group II 1.06 bird units per mg. Out of the 12 comparisons that were made between group I and II, eight of the pituitary glands from rats in group I contained more lactogen than those in group II, two were equal, and two contained less lactogen.

Upon assaying the pituitary glands in group I against those in group III it was found that the glands in group I contained 10.20 bird units per gland, those in group III contained 7.58 bird units per gland. This difference was due entirely to a greater lactogen concentration in the glands of group I, because the glands in group I were no larger than those in group III. Of the 12 comparisons that were made 9 of the pituitaries in group I contained more lactogen than those in group III, two were equal, and one contained less lactogen.

Twelve assay comparisons were made between groups II and III. The average results were as follows: group II, 9.71 bird units per pituitary gland and 1.09 bird units per mg. of pituitary tissue; and group III, 9.04 bird units per pituitary gland and 0.99 of a bird unit per mg. of pituitary tissue. Six of the pituitary glands from rats in group II contained more lactogen than those in group III, three were equal, and three contained less lactogen.

If one averages the assay results for each group in the two comparisons the following figures are obtained: group I, 10.52 bird units per pituitary gland and 1.17 bird units per mg. of pituitary tissue; group II, 9.59 bird units per gland and 1.08 bird units per mg. of pituitary tissue; and group III, 8.31 bird units per pituitary gland and 0.92 of a bird unit per mg. of pituitary tissue. These results are summarized in Table I.

DISCUSSION

In assay work of this type the question is frequently asked whether or not the hormone content of a gland is a reliable measure of the amount of hormone being discharged into the blood stream. With reference to the lactogenic hormone, the extensive assay results of Reece and Turner (2), with

TABLE I

Lactogen content of the pituitary gland of the lactating rat

No. of Animals	Group Number	Body wt. when sacrificed gm.	Pituitary wt. mg.	Bird units per pituitary gland	Bird units per mg. of pituitary tissue
12	I*	220	9.0	10.84	1.20
12	II*	244	8.9	9.46	1.06
12	I	220	9.0	10.20	1.13
12	III*	236	9.0	7.58	0.84
12	II	244	8.9	9.71	1.09
12	III	236	9.0	9.04	0.99
12**	I	220	9.0	10.52	1.17
12**	II	244	8.9	9.59	1.08
12**	III	236	9.0	8.31	0.92

{ Glands assayed in same group of pigeons.

* The pituitaries were taken 15 hrs. after the pups were removed from the females. In group I the pups were removed 36 hrs. after they were dropped. In group II they were removed 15 days after they were born. In group III they were removed 22 days after they were dropped.

** Average figures.

one exception, demonstrated that a pituitary gland with a high lactogen content is one that is actively discharging the hormone into the blood stream.

There was not a very marked difference in the lactogen content of pituitary glands taken from rats in three stages of the lactation cycle. It is possible that animal variability overshadows the influence of the stage of lactation on the lactogen content of the pituitary gland. If such is the case, animal variability can be ruled out only at a time when one is able to determine the amount of lactogen being excreted by the same animal over the entire lactation cycle.

The results secured show that the lactogen content of the pituitary gland was highest shortly after parturition. Following this time there was a gradual fall in the lactogen content of the pituitary gland until the end of the lactation period. A high pituitary lactogen content was not associated with the peak of the lactation curve and therefore suggests that the lactogenic hormone plays no important part in determining the height of the lactation curve. Inasmuch as there is a gradual fall in the pituitary lactogen content as the lactation cycle continues it seems probable that this hormone may be a factor in determining the rate of decline in milk production.

SUMMARY AND CONCLUSIONS

The pituitary glands collected from rats 51 hours after parturition contained 10.52 bird units per pituitary gland, those glands collected from rats 16 days following delivery contained 9.59 bird units per gland and those collected from rats 23 days after delivery contained 8.31 bird units.

On the basis of these results it is suggested that the lactogenic hormone plays no major rôle in determining the height of the lactation curve, but may be a factor in determining the rate of decline in milk production.

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THE SITE OF FAT SYNTHESIS IN THE MAMMARY GLAND[1]

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The organization of materials around the milk fat particle and the fact that the fat content of milk may be affected without influencing the amount of milk, suggests that milk fat is synthesized more or less independently from the other milk constituents. This naturally leads to the possible hypothesis that the milk fat may be synthesized in a different portion of the secretory epithelial cells. On this basis, work was begun first to establish the point of synthesis of milk fat and secondly to ascertain the mechanism of milk fat synthesis. To gain information on these points, three methods of attack were used: 1) stained fat was fed, 2) biochemical analyses of the gland were made, and 3) histochemical techniques were utilized. Reports on these studies follow in the order named.

PART ONE

FEEDING OF STAINED FAT

The work of Mendel and Daniels (7), and Gage and Fish (3), which give reviews of the previous literature, indicate that Sudan stained fat fed to lactating cows does not enter milk in its stained form. It is believed possible, however, since the stain was present in the blood after feeding that it might be found in the active secretory portion of the mammary gland. If this were the case, a histological study of such a gland would be of aid in the study of milk fat synthesis.

PROCEDURE

Two cows were fed butterfat or peanut oil which was previously stained with Sudan IV. One of these, a Jersey, was fed approximately 27 grams of the stain in a ten day feeding period. The other, a Holstein, ingested 15 grams of the Sudan IV in a nine day feeding period. Each cow was slaughtered at the end of her feeding period. Samples of milk were taken both before and during the feeding period. The carcass of each animal was inspected for the presence of Sudan stained adipose tissue. Each udder was immediately excised, and sections were placed in fixing solution for histochemical analysis. The remainder of the mammary tissue was frozen and kept in that condition until used for analysis.

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[1] A portion of the thesis submitted to the graduate school of the University of Minnesota by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy plus additional material from research at the University of Arkansas Agricultural Experiment Station.

[2] Now at the University of Arkansas.

[3] Paper No. 1671 Scientific Journal Series, Minnesota Agricultural Experiment Station. Research paper No. 627 Journal Series, University of Arkansas.

RESULTS

No definite evidences of the stain were detected in any of the body fat, udder tissue or milk fat. The adipose fats were a deep yellow color in each instance. There was no evidence to indicate that the adipose tissue of the body had been colored by the stain in any case. The udders were of normal color, and the fat globules of the alveoli did not appear to contain any of the stain. The milk fat was treated with various solvents in an effort to extract evidence of the presence of the stained fat, but the results were negative.

Attempts were made to separate the gland fat of the udder of the Jersey cow into fractions which would yield more intense color reactions. The alcoholic extract of the gland was of a deep yellow color. After filtering this extract and driving off the alcohol on an electric hot plate using a sand bath, ethyl ether and water were added and the two fractions were separated in a separatory funnel. The ether extract when concentrated was deep red in color. The water extract when concentrated was also deep red in color. When the latter was treated with very dilute sulphuric acid a red precipitate which was soluble in dilute ethyl alcohol floated to the top. For comparison an extract was made from the secretory tissue of another Jersey cow which was not fed stained fat. Here the concentrated ether extract was deeply colored, but instead of the deep red color noted in the water fraction in the gland of the Jersey cow which was fed the stained fat, this water fraction was almost colorless. Extracts of the milk fat from the cows fed the stained fat were saved both before and after the beginning of the fat feeding periods and treated in the same manner. The water extract in all four instances was colorless.

DISCUSSION OF RESULTS

This adds evidence to the possibility that the molecules of the stain become conjugated after they enter the body and become water soluble instead of fat soluble. This work was not carried further though it is interesting to note that the water soluble fractions, isolated from the udder of the cow fed stained fat, acted very similarly to the fractions worked on by Salent and Bengis (12) who worked with cats and rabbits, to study the metabolism of Sudan stains in the animal body.

While there may have been some difference in the color of the ether extracts from the two glands studied, the color was not considered positive evidence of the presence of the stains as various other pigments, such as carotene, were also present.

The work done fully corroborates the previous results of Mendel and Daniels (7), and Gage and Fish (3) that Sudan stained fat fed to lactating cows does not color the resulting milk fat which is produced. There was no definite evidence of the stain in the mammary gland. If present, the stain would be much diluted, and give a yellow rather than a red color.

The presence of natural pigments in the gland tissue would tend to cover up the color of the stain and render it indistinguishable from them.

The fact that the fat in the alveolus was unstained is significant. It is well known that blood fat is stained for some time after the feeding of stained fat. It is also well known that the blood lipides must to a large extent be the milk fat precursors. Since each cow in the experiments reported in this paper received at least one and one-half pounds of stained fat per day, the cows must have utilized some of the stained fat for the synthesis of milk fat. As the fat droplets visible in the alveoli were colorless, one can conclude that the changes which occur in the synthesis of milk fat from the blood precursors must take place before the formation of the fat droplets in the alveoli.

PART TWO

BIOCHEMICAL ANALYSIS

Review of the Literature

One of the authors, Petersen, Palmer and Eckles (9), had previously found that fat which they had extracted from two actively secreting bovine mammary glands had acid values of 8.07 and 11.30. Since acid value refers to the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in one gram of fat, these values indicated that a considerable amount of free fatty acid was present in the mammary gland fat. Preliminary work gave added indications that this was true, and it was decided to make a more detailed investigation.

PROCEDURE

Ten mammary glands in various stages of activity are reported in this study. Since the procedure varied somewhat, the various methods of extraction used will be mentioned in detail. All of the analyses were in duplicate or were the average of four analyses, and the necessary blanks were made. The results are given in Table 1.

Two extractions were made from the gland of Number 430, a Holstein, using different procedures.

First extraction: The udder tissue was frozen with liquid oxygen and ground in a mortar. It was extracted with a 3 to 1 ethyl alcohol-ethyl ether solution. This was evaporated almost to dryness and extracted with ethyl ether and petroleum ether. The ether was evaporated off in an air current, alcohol was added and the solution was titrated with aqueous sodium hydroxide using phenolphthalein as an indicator. An alcohol-ether extraction was made from 10 grams of the original tissue in a Soxhlet extractor. From the information obtained the acid value of the fat was calculated. These data are given in Table 1.

Second extraction: The frozen tissue was ground as finely as possible in a meat chopper, and mixed with five times its weight of plaster of Paris. A measured volume of ethyl ether was added to this mixture and shaken occasionally during the next few hours. After a final shaking, the mixture was allowed to settle, the clear extract at the top was decanted and its volume measured. This was called extraction Number 1. Two 25 cc. aliquots were titrated with aqueous sodium hydroxide. More ether was added to the calcium sulphate and udder tissue, and the same process was repeated for extraction Number 2. In this manner six extractions were made over a period of several days. When the final extraction was made, the first volume decanted was subtracted from the total volume of the ether added the first time in order to learn the volume of ether which remained in the calcium sulphate and udder tissue. This volume was considered as part of the final extraction. On the basis of these aliquots the total acidity of the extract could be calculated. This information, with that learned from the Soxhlet extractions, gave the necessary data for the calculations.

The second gland analyzed was ground as finely as possible with a meat chopper, and the tissue was extracted with small amounts of ethyl alcohol and later ethyl ether. These combined extracts were extracted with ethyl ether in a separatory funnel. The ether extract was placed in an evaporating dish, and the ether was blown off with an electric fan. The dried fat was purified with benzene, it was filtered, the benzene was driven off, and the weight of the fat was obtained. It was dissolved in equal parts of benzene and ethyl alcohol and titrated with aqueous sodium hydroxide using phenolphthalein as the indicator.

After grinding with a meat chopper, the remainder of the glands analyzed were mixed with five times their weight of anhydrous calcium sulphate and extracted in a continuous extractor with ethyl ether. The fat obtained was taken up in either benzene or petroleum ether. Weights and titration values were obtained using the same reagent and indicators as in the previous analyses.

RESULTS

A summary of the analyses is listed below:

TABLE 1

Analysis of the fat extracted from bovine mammary glands

Cow No.	Acid value	Remarks
430	18.63	End of a prolonged lactation period.
7	13.21	Dry gland.
82	38.20	Actively secreting gland from a cow which had been fresh two months.
5	37.58	In active secretion, but tissue of unknown origin from a slaughter house.
3	18.11	Dry gland from a shorthorn cow which would have freshened in about 2 weeks.
6	17.90	Dry gland from a non-pregnant cow which had been dry 8 months.
73	22.84	Dry gland from a cow milked four months and dried without further milking. Was dry 27 days before slaughter.
Mixture of 3 glands	.72	Slaughterhouse tissue from the glands of a yearling heifer which was not carrying a calf.

DISCUSSION OF RESULTS

A study of the data in Table 1 points to a close relationship between the stage of activity of the gland and the amount of free fatty acid which is present. Glands which have not undergone the development of pregnancy contain only a trace of this material. The acid values of the fat from the secretory tissue of glands which had previously been in active secretion, but which were in a resting state at the time of analysis, varied from 13.21 to 22.84. Two actively secreting glands had acid values of 38.20 and 37.58, while a third gland from a cow which was at the end of prolonged lactation period yielded an acid value of 18.63. Neither blood fat, which must to some extent be the precursor of milk fat, nor milk fat which is the finished product of milk fat synthesis, contain appreciable amounts of free fatty acid. The fact that there is a close relationship between the stage of activity of the gland and the amount of this material present in the secretory tissue, and also that such comparatively large amounts are present during active secretion suggests that free fatty acids are involved in the actual process of milk fat synthesis.

PART THREE

HISTOCHEMICAL ANALYSIS

As the preceding biochemical studies had indicated the presence of free fatty acids in the mammary gland fat, histochemical studies were undertaken in the hope of ascertaining where these fatty acids were located in the secretory tissue. Since the specificity of any one stain differentiating neutral fat from free fatty acids is open to question, five different stains were used. The five stains used were: Neutral red, brilliant cresyl blue, nile blue sulphate, Oil Red O, and osmic acid.

Neutral red was chosen as authorities cited by Langeron (5) claim it to stain neutral fats a faint yellow and fatty acids a deep red. This dye also acts as an indicator changing to a red at a pH of 6.8 and to a yellow at a pH of 8.0.

Brilliant cresyl blue was used as the small amount of literature which is also cited by Langeron (5) indicates a general agreement that brilliant cresyl blue has use as a fat stain.

The literature in regard to nile blue sulphate, another of the stains used, is somewhat larger and more controversial. Langeron (5), Lison (6), and Kay and Whitehead (13) have written detailed discussions in regard to the use of this stain. Most are agreed that the red color is quite specific for neutral fats though the blue stain is not as specific in designating free fatty acids.

Oil Red O, another stain selected for use, was found by French (2) and Proescher (10) to give superior results to Sudan III to which it is chemically very similar. This is a general fat stain which stains neutral fats more deeply than fatty acids and sterol esters.

Lison (6) takes exception to the view that Sudan is a differential stain because it adheres to the fats by the phenomenon of simple absorption of the stain by the fat. He maintains that, since this is true, it cannot be used as a differential stain. A similar criticism could not be applied against the use of the stain in alkaline solution. It was noted in making the photomicrographs that the alkaline stain appeared to give differentiation where the neutral stain did not. One of the difficulties in its use is that it is soluble only in solvents which tend to dissolve out the fat. In this work the method of Gross (4) was followed in using a 50 per cent diacetin solution in water as a solvent for the dye.

The various groups of substances differ in their readiness to be oxidized by osmic acid thus causing differences both in the rapidity of staining and in the color produced. Natural fats are blackened the most deeply. Langeron (5) and Lison (6) discuss the use of this stain as a fat stain. Since it stains other compounds besides the fats which are present, extreme care must be taken in interpreting the results obtained from its use. However, since biochemical analysis has shown large amounts of the free fatty acids to be present and as other stains were also to be used, osmic acid was believed to be of possible value in this study.

The method of Romiew (11) was used for detection of cholesterol and sterol esters. This method according to Lison (6) is specific for the detection of these substances.

A test for phospholipides was also made based on the well known fact that these substances are not soluble in acetone which is a solvent for other types of fats.

EXPERIMENTAL PROCEDURE

Portions of the secretory tissue of an active mammary gland were placed in a solution consisting of 10 parts formalin and 90 parts Locke's physiological saline solution. They remained in this solution from 2 to 4 days. The time element was kept as short as possible since Millot and Giberton (8) have shown that too long preservation in formol solution alters the amount of free fatty acid in the tissues. The photographs and drawings were prepared from this material.

Wherever possible the stains were prepared as aqueous solutions. Neutral red and brilliant cresyl blue were made up as 1 per cent aqueous solutions. A 1 per cent aqueous solution of nile blue sulphate was hydrolized with a few drops of sulphuric acid after the method described by Conn (1). Sections of tissue were also stained in the gas fumes of a 2 per cent solution of osmic acid.

EXPERIMENTAL RESULTS

A drawing of portions of the walls of two alveoli with a blood capillary between which was stained with neutral red is shown in Fig. 1. The deep

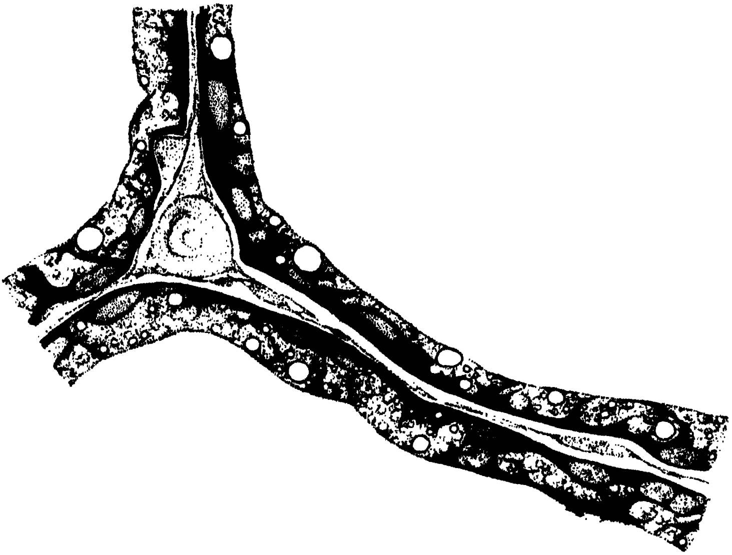

FIG. 1. A portion of the walls of two alveoli stained with neutral red. The free fatty acids are deeply stained while the globules of neutral fat are unstained.

stain represents free fatty acids with the uncolored fat globules as neutral fat. It will be noted that there is a greater concentration of free fatty acid at the base of the secretory cells and a greater concentration of neutral fat in the distal portion.

Figure 2 is a drawing of a section stained with brilliant cresyl blue. The deeper stained free fatty acids and uncolored fat globules are shown

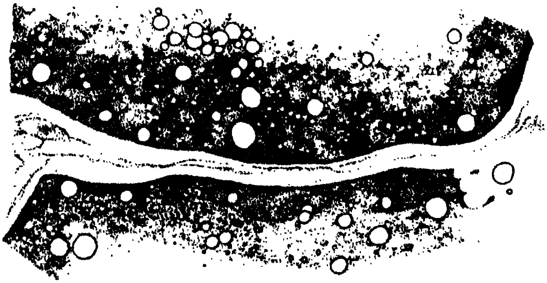

FIG. 2. A section stained with brilliant cresyl blue. The free fatty acids are deeply stained and the fat globules unstained.

in essentially the same position as that indicated where neutral red was used as the fat stain.

When stained with nile blue sulphate (Fig. 3) the same picture is again

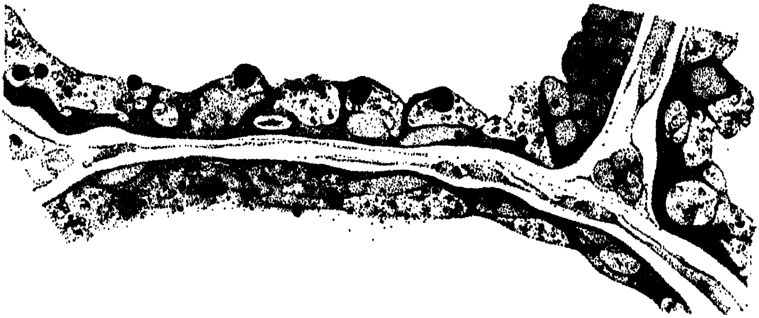

FIG. 3. A section stained with nile blue sulphate. The free fatty acids are deeply stained while the globules of neutral fat are unstained.

represented with the free fatty acid in the deeply stained area and neutral fat in the form of fat globules.

Figure 4 is a drawing of a section stained with Oil Red O. The deepest stain is taken up by the neutral fats which are located in the same area as that indicated by the previous figures where other stains were used. Since this is a general fat stain, staining most types of fats, other fatty substances besides neutral fats and free fatty acids are detected by its use, but it can be observed that besides the neutral fats the epithelial tissue contains other fatty substances. A large portion of this material could consist of free fatty acids from the basis of the observations on the previous sections where other stains were used.

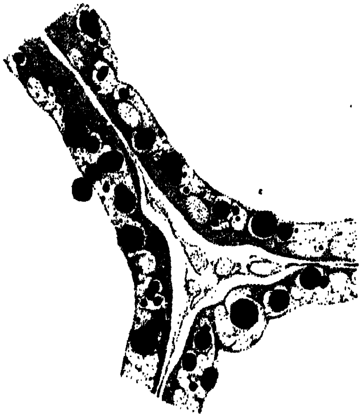

FIG. 4. A section stained with Oil Red O. The neutral fats take the deepest stain and other fatty substances are less intensely colored.

A comparable picture is obtained when the section is stained with osmic acid. See Figure 5. The deepest stained globules, the neutral fats, are

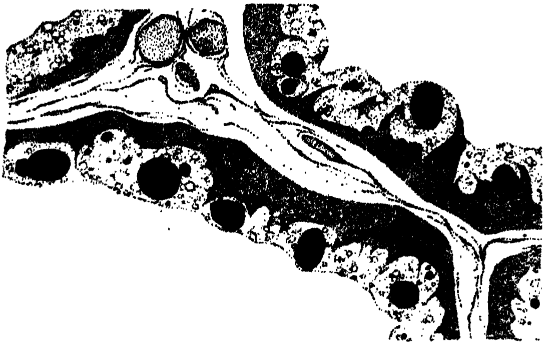

FIG. 5. A section stained with osmic acid. The globules of neutral fat are stained deeply while other fatty constituents are less deeply stained (indicated by stippling).

located in the same areas as in the previous figures. The areas which stained brown (stippled) indicated that other fatty constituents were present.

Photomicrographs were also made for comparison with the drawings.

The Romiew stain, used to indicate the presence of cholesterol and sterol esters, indicated concentrations of these materials in all of the epithelium adjacent to the alveolus.

The results of the technique used to indicate the presence of phospholipides were almost negative. After the extraction with acetone, the tissue took up only a slight amount of osmic acid giving the section a uniform light brown color.

DISCUSSION OF RESULTS

Nile blue sulphate, brilliant cresyl blue, and neutral red, the three stains used for differentiating between free fatty acids and neutral fat, gave the same picture; namely, a positive stain for free fatty acids and for neutral fat. The free fatty acid was distributed throughout the epithelial tissue with the greater concentrations in the basal portions of the cells, while the neutral fats were concentrated to the larger extent in the distal portion of the cells.

Osmic acid and Oil Red O indicate neutral fats to be located in the same position as that indicated by the three previously mentioned stains. While neither osmic acid nor Oil Red O indicate free fatty acid, specifically, they do indicate that there is a concentration of fatty constituents of some type, other than neutral fat, in the secretory epithelium.

Since there was never any evidence that the free fatty acids were present in the form of particles, it is suggested that the free fatty acids are present in either molecular or colloidal state. As they are synthesized into neutral fats there is a change in interfacial tension, causing the particles to coalesce so that at the lumen they are of normal milk particle size.

On the basis of the acid value secured for the total fat of the gland, there are sufficient free fatty acids present to account for the staining picture when such fatty acids are largely limited to the basal portions of the secretory epithelium.

SUMMARY AND CONCLUSIONS

1. An attempt to trace the course of milk fat synthesis by the use of feeds stained with Sudan stain gave negative results partly because of the apparent changing of the structure of the dye molecule to a water soluble compound.

2. Biochemical studies indicated the presence of quite large amounts of free fatty acids in actively secreting glands. They pointed to a close relationship between the stage of activity and the amount of free fatty acid which was present, with resting glands containing much less of this material than do actively secreting glands. Glands undeveloped by pregnancy contain only a trace of free fatty acid.

3. Histochemical studies with nile blue sulphate, brilliant cresyl blue, and neutral red indicated the secretory cells of the mammary gland particularly in their basal portions, contained free fatty acids. Neutral fats were located near the distal portions of the alveolus. Oil Red O and osmic acid added confirmatory evidence to the results of the previously mentioned stains.

4. It is suggested from the evidence presented that the free fatty acids in the basal portions of the secretory epithelium are present in either molecular or colloidal state.

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RELATION OF VITAMIN C, LECITHIN, AND CAROTENE OF MILK TO THE DEVELOPMENT OF OXIDIZED FLAVOR[1]

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INTRODUCTION

Oxidized flavor is one of the most objectionable off-flavors of milk and one of the most difficult to control. During recent years, in face of greater sanitary precautions, and increased use of pasteurization, oxidized flavored milk has become increasingly prevalent. Increased handling and low bacterial counts seem to favor the development of oxidized flavor. Outbreaks of oxidized flavored milk have been particularly intensive in dairies producing high quality milk, especially if the interval between production and consumption is longer than usual.

Oxidized flavor has been studied rather extensively during the last ten years. Out of these studies has come the knowledge that it is a developed off-flavor that may be catalyzed in various ways such as: exposure to light rays, metal contamination, and heat. In accordance with these investigations, plant operators have taken special precautions in handling milk. Little trouble has been reported during the seasons of the year when pasture and other green feeds are plentiful. During the winter season, however, some milk develops oxidized flavor even when produced under the most sanitary conditions and with the greatest protective efforts against metal contamination and exposure to light. Extreme precautionary measures during the processing and handling operations do not always prevent its occurrence. The production of milk which is relatively non-susceptible to oxidation may be a possible solution of the problem. It seemed desirable, therefore, to study the raw milk from individual cows in an effort to determine any possible relationship of certain milk constituents to the oxidation process.

REVIEW OF LITERATURE

Evidence has been presented that lecithin rather than butterfat is involved in the development of oxidized flavor (11, 12, 13). Some investigators (3, 4, 9) have reported a possible relation between oxidized flavor and the vitamin C in milk.

According to Guthrie and Brueckner (6) there is no relation between

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[1] Contribution No. 124 from the Department of Dairy Husbandry and No. 235 from the Department of Chemistry.

[2] The data presented in this paper are from a thesis submitted to the Graduate School of the Kansas State College in partial fulfillment of the degree of Master of Science, 1938.

oxidized flavor and the breed, period of lactation, or age of cows. However, Stebnitz and Sommer (10) found that the butterfat in the milk from Holstein cows was more susceptible to oxidation than that from any other breed, when on winter rations. Milk fat from Guernsey cows had an exceptionally high stability value or peroxide number.

Some of the earliest work relating to feed and oxidized flavor was done by Kende (8) in 1932. His theory for the absence of oxidized flavor in summer milk was that green feeds and fresh hay contain considerable amounts of reducing substances which tend to prevent oxidized flavor. Furthermore, that green feeds seem to lose this reducing substance during storage because of auto-oxidation. In agreement with Kende's observation Brown, Thurston, and Dustman (3) found that by changing cows from dry feed to dry feed plus pasture the milk became non-susceptible to oxidized flavor.

Anderson (1) has shown that carotene may be the substance in green feed which retards the development of oxidized flavor. He found that by supplementing the regular winter ration with five pounds of carrots or five pounds of machine cured hay, oxidized flavor could be eliminated from the milk of a cow that had previously produced oxidized milk. Since these feeds are high in carotene he attributed the improvement in flavor to this substance.

The only indication found by Guthrie and Brueckner (6) of a relation between the feed of the cow and oxidized flavor was the tendency for most cows which produced such milk in the winter not to produce milk that acquired this off-flavor in the summer. They concluded that dry feed was not the sole cause of the development of oxidized flavor since there was a variation in intensity of off-flavor developed in the milk from different quarters of the udder.

Stebnitz and Sommer (10) found that the stability value of fat toward oxidation decreased when cows went on grass as compared with winter rations. According to their results, fat from cows receiving grass as part of their ration is less saturated and therefore more susceptible to oxidation. Yet they found that the milk did not oxidize when the cows were on green feed. They concluded that there are protecting substances in milk in increased amounts when cows are on grass which prevent the development of oxidized flavor. They were unable to find any relation, however, between the carotene in the milk, as indicated by color of fat, and the stability of milk toward oxidation.

EXPERIMENTAL PROCEDURE

This study was undertaken to obtain more information on the occurrence of oxidized flavor in raw milk and its relation to the vitamin C, lecithin, and carotene (as indicated by color of the milk fat) of the milk.

Milk from four breeds of dairy cows in the college herd was studied. The cows used were fed a grain mixture in addition to medium grade alfalfa hay and sorgo silage. They did not receive pasture during the trial, except during the month of May. A group of Holstein cows that were on a special feeding trial were also included in this study. These cows differed from the other cows in that they were receiving prairie hay instead of alfalfa and had never received any green feed since they were six months old. This group will be spoken of as E-Holsteins throughout this paper.

On three consecutive days of each month, samples of morning milk were collected from each of the 60–70 cows in the herd. The samples were collected directly from an aluminum milking pail and placed in glass bottles. These samples were divided into two parts, one part being scored immediately for flavor and tested for vitamin C, while the other part was stored three days at 45° F. before being scored for flavor and tested for vitamin C.

The milk was examined organoleptically by two judges for off-flavors. A system of numbering was used to conceal the identity of the samples from the judges. The intensity of the oxidized flavor was indicated by numbers ranging from one (slightly oxidized) to five (very pronounced). All other off-flavors were recorded, but were not given a rating according to intensity.

Vitamin C determinations were made according to the method developed by Bessey and King (2) and modified by Whitnah and Riddell (14). These tests were made on both the fresh milk and the stored milk, for all samples taken during November to March inclusive.

Horralls' (7) method of determining lecithin in milk was used. Only the amounts of phosphorus in p.p.m. were calculated. The actual amount of lecithin could be determined by multiplying a constant derived from the empirical formula of lecithin (7) with p.p.m. of phosphorus. However, the p.p.m. of phosphorus in an ether extraction of fat is representative of the amount of lecithin in the sample. Lecithin determinations were made on three day composites of milk collected in December and March.

The method used for determining the amount of color in milk fat was developed by Whitnah[3] and used as a relative index of carotene. This method involves the comparison of a clear fat solution with a standard 0.1 per cent potassium dichromate solution in a Klette colorimeter. Color determinations were made on three day composites of milk collected monthly during January to May.

Two feeding trials were conducted in which the regular winter rations of animals that had been producing badly oxidized milk were supplemented with feeds high in carotene. One feed was a carotene concentrate, soluble in fat solvents, and the other was dehydrated green oats. The carotene

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[3] Unpublished data by C. H. Whitnah of the Department of Chemistry, Kansas State College.

TABLE 1

Comparison of breeds of cows in frequency of occurrence of oxidized flavor in milk samples collected monthly, November to May

Breed	Nov.		Dec.		Jan.		Feb.		Mar.		Apr.		May		Total	
	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized
Jersey	13	0	13	7.7	48	14.6	48	6.3	48	8.3	48	2.1	45	0	263	6.1
Guernsey	11	18.1	12	33.3	42	21.4	39	2.5	38	2.6	36	0	39	0	217	7.8
Ayrshire	12	16.7	14	28.6	39	30.7	39	5.1	35	22.9	27	22.0	30	13.3	196	19.4
Holstein	14	21.4	16	31.3	48	31.3	39	20.5	44	9.1	46	8.7	45	2.2	252	15.9
E-Holstein	9	11.1	9	0	30	0	36	5.5	32	18.7	38	7.9	45	2.2	199	6.5
Total	59	13.6	64	21.9	207	20.8	201	8.0	197	11.7	195	7.2	204	2.9	1127	11.0

concentrate contained 150 mg. of carotene per pound, and the dehydrated green oats contained 103 mg. of carotene per pound.

In the first trial eight cows were selected, four of which were fed a carotene supplement while the other four were continued as controls on the regular winter ration without supplement. Three cows were fed two pounds per head daily of dehydrated oats and the fourth cow received four pounds daily of the fat soluble concentrate. In the second trial, two cows were fed the fat soluble concentrate at the rate of one pound per head daily, while two cows were continued as before without any supplement.

Both feeding trials lasted ten days. During the trials, samples of milk were collected daily and examined for flavor defects and analyzed for color.

EXPERIMENTAL RESULTS

Table 1 shows the number of samples analyzed each month by breeds and the percentage which developed oxidized flavor. Off-flavors found, other than oxidized were: rancid, salty, feedy, and storage. No significant relation was found between oxidized flavor and any other off-flavor.

Oxidized flavor was detected in 11 per cent of 1,127 samples examined during the regular monthly herd test (Table 1). There was a decided decrease in this flavor defect during the month of May. This improvement in flavor corresponded with the time when the cows received their first pasture after having been fed a winter ration for six months.

Oxidized flavor occurred in 17.4 per cent of all Ayrshire and Holstein samples considered together, whereas it occurred in only 6.9 per cent of all Jersey and Guernsey samples considered together. The E–Holstein cows were as free from the oxidized flavor as the Jersey and Guernsey breeds. The milk produced by this group, however, frequently became so rancid that other off-flavors might have been masked.

Relation of Vitamin C to Oxidized Flavor

The relation of oxidized flavor in milk to both the vitamin C content of fresh milk and the amount of vitamin C lost during a three day storage period is shown in Table 2. The mean vitamin C content of fresh milk is practically the same in the oxidized milks as in the non-oxidized milks. For the Jersey, Guernsey, and Ayrshire breeds, there was a greater loss of vitamin C in the oxidized flavored milks than in the non-oxidized group, yet the reverse is true in the Holstein breed. The correlation coefficients between the vitamin C content of fresh milk and the oxidized flavor rating for 732 samples was only 0.017. The coefficient between the amount of vitamin C lost in three days storage of milk and the oxidized flavor rating was 0.109, which though slightly higher was not significant.

Relation of Lecithin to Oxidized Flavor

The lecithin content, expressed as parts per million of phosphorus, and the prevalence of oxidized flavor in various lecithin intervals are presented

TABLE 2

Relation between the occurrence of oxidized flavor and the vitamin C content of fresh milk, and the amount of vitamin C lost during 3-day storage

Breed	No. samples	Mean vitamin C p.p.m.				Correlation coefficient*	
		Original		3-day storage loss		Original vitamin C	3-day storage loss
		Oxidized	Non-oxidized	Oxidized	Non-oxidized		
Jersey	170	24.7	24.3	14.0	8.9	0.042	0.017
Guernsey	142	21.5	21.8	10.6	8.7	– 0.003	0.010
Ayrshire	141	20.5	19.7	10.7	8.3	0.142	0.016
Holstein	165	17.9	18.5	6.8	8.1	0.067	0.003
E.Holstein	114	17.5	19.0	6.8	7.9	0.009	0.009
All samples	732	20.1	20.4	10.3	8.9	0.017	0.109

* Computed by the Product-Moment method using Vitamin C in p.p.m. as compared with an oxidized flavor rating of 0–5.

in Table 3. The occurrence of oxidized flavor was only slightly more frequent in the milks with the lower lecithin contents. The correlation coefficient between lecithin and the oxidized flavor rating was 0.0056 for 122 samples. Such a low coefficient indicates no significant relation.

TABLE 3

Prevalence of oxidized flavor in milks grouped according to lecithin content

Class interval	No. of samples			Per cent of total			Per cent of Int. Tot.		
Phosphorus p.p.m.	Oxidized	Non-oxidized	Total	Oxidized	Non-oxidized	Total	Oxidized	Non-oxidized	Total
39.5– 69.5	11	36	47	47.8	37.1	39.2	23.4	76.6	100.0
69.5– 99.5	4	15	19	12.4	15.5	15.8	21.1	78.9	100.0
99.5–129.5	3	19	22	13.0	19.6	18.3	13.6	86.4	100.0
129.5–159.5	3	10	13	13.0	10.3	10.8	23.1	76.9	100.0
159.5–189.5	1	9	10	4.4	9.3	8.3	10.0	90.0	100.0
189.5–219.5	0	4	4	0.0	4.1	3.3	0.0	0.0	0.0
219.5–249.5	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
249.5–279.5	1	4	5	4.4	4.1	4.2	20.0	80.0	100.0
Total	23	97	120	100.0	100.0	100.0	20.5	79.5	100.0

Effect of Carotene Supplements on Oxidized Flavor

The results of the first feeding trial are summarized in Table 5 and Figure 1. After receiving 600 mgms. of carotene daily for two days, cow number 134 produced milk that gave no indication of developing an oxidized flavor, although she had been consistently producing milk with this flavor defect. Her milk had an exceptionally good flavor after five or six days of supplemented feeding. The other three cows (numbers 370, 130 and 242), after receiving 206 mgms. of carotene per head daily for 3 days, produced milk that did not develop oxidized flavor. Although the oxidized flavor seemed to be eliminated from the milk of these three cows the flavor was not as good as it was for cow number 134 which received the higher amount of carotene On the ninth day of the trial cow number 130, which was on the lower carotene level, produced milk which developed a slightly oxidized flavor. This was the only instance, in the milk of all four cows, in which oxidized flavor returned after the third day of the trial. The control group of four cows maintained on an unsupplemented ration produced oxidized milk during the entire feeding trial (Figure 1)

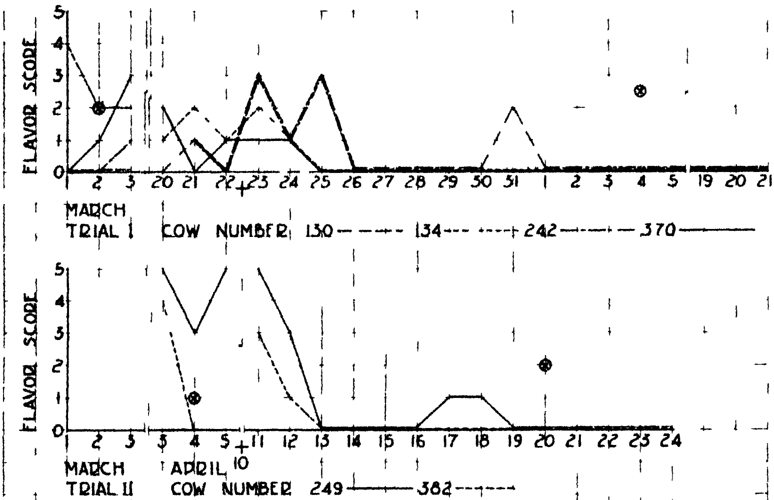

FIG 1-EFFECT OF CAROTENE SUPPLEMENTS IN THE RATION ON OXIDIZED FLAVOR

⊗ INDICATES FLAVOR SCORE OF UNSUPPLEMENTED CONTROLS

+ DATE CAROTENE SUPPLEMENT WAS STARTED

In the second trial cows 249 and 382 received 150 mgms of carotene per head daily. As shown by the results in Table 6 and Figure 1 the flavor was improved after the second day of carotene feeding The improvement, in the milk of these cows, was not so pronounced as from the cows in the first feeding trial on a higher level of carotene. Cow number 249 produced milk with a slightly oxidized flavor on the seventh and eight days. Milk from both cows was slightly off-flavored (stale or musty) during the entire feeding trial. When they were turned out to pasture a decided flavor improvement was noticed. The control group continued to produce milk with an oxidized flavor during the entire feeding trial (Figure 1).

No attempt was made to learn how soon oxidized flavor would have returned in the milk of the cows that had been receiving a carotene supplement. All of the animals used in this experiment were put on pasture shortly after the feeding trials ended.

Relation of Milk Fat Color to Oxidized Flavor

The relation of milk fat to oxidized flavor is presented in Table 5. Since the Holstein and Ayrshire breeds do not have such highly colored milk as the Jersey and Guernsey breeds, the results were computed separately. The consistently lower mean color averages for the milks having oxidized flavor as compared with the non-oxidized milks indicate that this flavor defect occurs more frequently in low colored milk. In the 27 samples of milks in the oxidized group (Table 5) only four were above breed average in color. No oxidized samples of Jersey and Guernsey milk were above breed average in color.

TABLE 4

Intensity of oxidized flavor in the milk of cows prior to and after feeding carotene supplement

Date	Oxidized flavor rating*												
	Trial Number 1								Date	Trial Number 2			
	Cows fed supplement				Control cows					Cows fed supplement		Control cows	
	370	134	130	242	249	136	271	E 38		249	382	220	374
	Winter feed with no carotene supplement												
Mar 1	0	4	0	0	5	3	1	1	Mar. 1	5	0	2	0
" 2	1	2	0	1	5	1	1	2	" 2	5	0	3	2
" 3	3	1	1	0	5	0	0	0	" 3	5	0	0	0
" 20	1	1	0	0	2	0	0	3	Apr 3	5	4	0	0
" 21	0	2	1	1	0	2	0	2	" 4	3	0	1	0
" 22	1	1	0	0	0	3	1	0	" 5	5	0	0	1
	Supplement† started March 22								Supplement‡ started April 10				
Mar. 23	1	2	3	3					Apr. 11	5	3		
" 24	1	1	1	1					" 12	3	1		
" 25	0	0	3	3					" 13	0	0		
" 26	0	0	0	0					" 14	0	0		
" 27	0	0	0	0					" 15	0	0		
" 28	0	0	0	0					" 16	0	0		
" 29	0	0	0	0					" 17	1	0		
" 30	0	0	0	0					" 18	1	0		
" 31	0	0	2	0					" 19	0	0		
Apr. 1	0	0	0	0					" 20	0	0	1	2
" 2	0	0	0	0					" 21	0	0	0	3
" 3	0	0	0	0	5	0	2	1	" 22	0	0	3	3
" 4	0	0	0	0	3	3	4	0	" 23	0	0		
" 5	0	0	0	0	5	2	1	2	" 24	0	0		

* Oxidized flavor rating from 0, which was free from this flavor defect to 5 which was very pronounced.

† Cows 370, 130, and 242 received 2 pounds per head daily of dehydrated green oats containing 103 mg. of carotene per pound. Cow 134 received 4 pounds daily of a fat solvent soluble concentrate containing 150 mgs. of carotene per pound.

‡ Cows 249 and 382 received 1 pound per head daily of the fat soluble, concentrate.

As a further indication that oxidized flavor occurs more frequently in milks of low color intensity it will be noted (Table 1) as mentioned previously in this paper, that oxidized flavor occurred in 2.5 times more samples of Holstein and Ayrshire milk than it did in Jersey and Guernsey milk. Since the Holstein and Ayrshire breeds are known to give milk of low color, this may possibly explain the breed difference in prevalence of oxidized flavor.

TABLE 5

The comparison of color intensity of milk developing oxidized flavor with milk free from this off-flavor

Flavor grouping	Color score									
	Feb.		Mar.		Apr.		May		Total	
	No. samples	Mean	No. samples	Mean	No. samples	Mean	No. samples	Mean	No. samples	Mean
Jersey-Guernsey milk										
Non-oxidized	25	15.7	25	14.9	27	14.1	28	50.7	105	25.2
Oxidized	3	13.5	3	7.3	1	3.7	0	0.0	7	9.4
Holstein-Ayrshire milk										
Non-oxidized	21	7.9	21	8.9	18	10.3	23	26.7	83	13.8
Oxidized	5	7.1	6	8.4	7	7.9	2	8.2	20	7.9

Color determinations were made on the milk fat of each cow during the feeding trials. These determinations are not shown for the cows in the second feeding trial because several of the samples were accidentally destroyed.

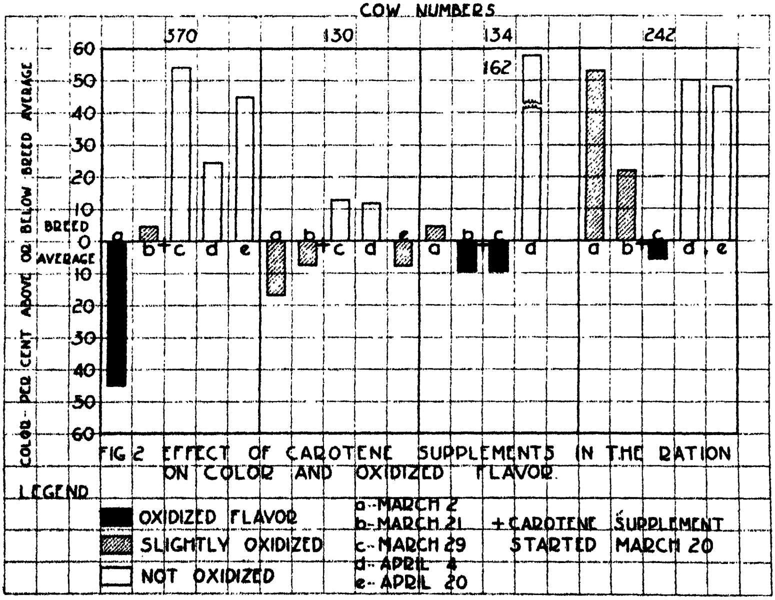

FIG 2 EFFECT OF CAROTENE SUPPLEMENTS IN THE RATION ON COLOR AND OXIDIZED FLAVOR.

Table 6 and Figure 2 show that there was an appreciable rise in fat color intensity shortly after the cows had received a carotene supplement. In each case oxidized flavor was eliminated as the color intensity increased. Color intensity was increased as much as 162 per cent above breed average after a carotene supplement had been fed (Cow number 134, Table 6, Figure 2). This cow received nearly 400 mgms. more carotene per day than did the other cows in the same group. High color intensity, in comparison with the breed average, did not always prevent the development of oxidized flavor. This was shown by the fact that milk from cow number 242 was slightly oxidized when the color intensity of the milk fat was 53 per cent above the breed average. Her milk, however, was decreasing in color and at the same time the off-flavor was becoming more pronounced. The flavor defect was eliminated entirely after feeding a carotene supplement.

TABLE 6

Effect of carotene supplements to the ration on color intensity of milk fat, and the development of oxidized flavor in milk

Date	Cow number											
	370			130			134			242		
	Color score	Per cent of breed ave.*	Flavor score†	Color score	Per cent of breed ave.*	Flavor score†	Color score	Per cent of breed ave.*	Flavor score†	Color score	Per cent of breed ave.*	Flavor score†
Mar. 2	5.7	45	2	8.4	85	1	10.3	105	1	11.8	153	1
" 21	13.0	105	1	9.5	93	1	9.5	93	2	9.4	122	1
Ration supplemented with a carotene concentrate Mar. 22												
Mar. 29	18.0	154	0	12.0	113	0	9.3	93	0	7.5	95	2
Apr. 4	14.7	124	0	11.9	112	0	27.8	262	0	10.1	150	0
" 20	17.0	145	0	10.2	96	1	......	....	...	8.7	148	0

* Breed average was obtained in each instance by averaging the color score for all individuals within the herd.

† A score of 0 to 5 was used to indicate the intensity of the oxidized flavor.

DISCUSSION

The prevalence of oxidized flavor in raw milk does not seem to be affected by the original amount of vitamin C in the milk or the amount of vitamin C lost during 3 days storage, Chilson (4) found that all the vitamin C was destroyed by the time oxidized flavor was detectable. In this trial, however, there were instances in which most of the vitamin C was left after 3 days storage, yet oxidized flavor had developed. Sharp, Trout, and Guthrie (9) found a definite relationship between the rate of disappearance of vitamin C during storage and the development of oxidized flavor when the milk was pasteurized at 143° F. for 30 minutes. Such a relationship may exist in pasteurized milk but it did not hold true for the data herein reported for raw milk.

From the review of the literature it seemed that lecithin was the substance oxidized when milk developed this off-flavor. Consequently it would appear that the amount of lecithin in the milk would influence the intensity and frequency of oxidation. In this trial, however, oxidized flavor occurred just as frequently in the milks of lower lecithin content as in the milks of the higher content. Thurston and co-workers (13) found that the physical state of lecithin in the milk, *i.e.*, whether it surrounds the fat globules or is dispersed in the plasma, was a contributing factor in causing oxidation. If lecithin is a cause of oxidized flavor it would seem that the physical or the chemical state is more important than the amount.

Results obtained in this trial seem to indicate that there is a relationship between breed and susceptibility of raw milk to becoming oxidized. Guthrie and Brueckner (6) and Dahle and Palmer (5) could find no relationship, but the results obtained by Stebnitz and Sommer (10) agree with the foregoing results. There was a significant difference when Holstein and Ayrshire breeds considered together were compared with the Jersey and Guernsey breeds, considered together. With the exception of the E-Holsteins oxidized flavor occurred in 15.8–19.4 per cent of 448 samples of Holstein and Ayrshire milk, whereas it occurred in only 6.1–7.8 per cent of 480 samples of Jersey and Guernsey milk.

Results of this experiment seem to indicate that high color intensity of the milk fat renders the milk less susceptible to the development of oxidized flavor. Since the Jersey and Guernsey breeds produced milk comparatively free from oxidized flavor, and since it is known that these breeds put more color into their milk than other breeds it might be expected that oxidized flavor would be less prevalent in highly colored milk. From observations made on milk from the entire herd, oxidized flavor occurred more frequently in the milk that was below breed average in fat color. With but four exceptions in 27 oxidized samples of milk all of the oxidized samples were below breed average in color intensity. Some exceptions might be expected in view of the many factors known to cause oxidized flavor.

Oxidized flavor seldom occurs during the seasons of the year when the cows are receiving plenty of green feed or a relatively high carotene intake from their ration. The susceptibility of raw milk from cows on winter rations to become oxidized can be eliminated by supplying a carotene supplement in the feed of the cow. A carotene concentrate supplying as little as 206 mgms. of carotene daily proved effective in preventing oxidized flavor in the milk of cows that had been consistently producing milk that became oxidized. When a lower amount of carotene was fed it decreased the intensity of oxidized flavor, but was insufficient to produce normal flavored milk. There was a decided increase in the fat color of the milk produced by cows receiving a carotene supplement indicating that the increased carotene was directly or indirectly responsible for obviating oxidized flavor.

It appears from the results in this experiment, that the problem of obviating oxidized flavor in raw milk is a matter of producing milk relatively non-susceptible to oxidation. This can be done by feeding a ration high in carotene throughout the year or by the use of supplements of carotene concentrates during winter months.

SUMMARY AND CONCLUSIONS

1. Oxidized flavor occurred in 11 per cent of 1,127 samples of milk.

2. No relation was found between the amount of vitamin C in the original milk, or between the amount of vitamin C lost during 3 days storage, and the development of oxidized flavor.

3. No relation was found between the frequency of occurrence of oxidized flavor and the lecithin content of the milk.

4. There appeared to be a relationship between color intensity of milk fat, as produced by different breeds and the development of oxidized flavor. Oxidized flavor occurred in 6.1–7.8 per cent, respectively, of 480 samples of Jersey and Guernsey milk and in 15.8–19.4 per cent, respectively of 448 samples of Holstein and Ayrshire milk.

5. Oxidized flavor was more prevalent in milks that were below breed average in fat color intensity.

6. The development of oxidized flavor in raw milk was effectively prevented by feeding as little as 206 mgms. of carotene per head daily to cows that had been consistently producing milk with this off-flavor.

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OXIDIZED FLAVOR IN MILK. VI. A STUDY OF THE RELATION OF TITRATABLE ACIDITY TO METAL-DEVELOPED OXIDIZED FLAVOR IN MILK[1]

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The development of oxidized flavor in high-quality milk has been one of the most harassing problems which confront the milk industry. Many investigators have spent much time seeking the cause and remedy of this off-flavor.

Recently Anderson, Dowd, and Stuewer (1) found that the titratable acidity of winter milk is higher than that of summer milk. This seasonal change is coincidental with the change in susceptibility to oxidized flavor. It seems possible that this condition may have been partially responsible for oxidized flavor. Anderson and his co-workers found an association between acidity and the tendency to develop this flavor. They were unable to eliminate the defect by giving a new coating of tin to all equipment not properly tinned or made of stainless steel. However, they were able to correct this defect by lowering the apparent acidity of the milk to 0.145 per cent in pasteurized milk. As a result of their work they concluded that this defect was associated with the apparent acidity of the milk and that milk of high acidity invariably developed an oxidized flavor upon pasteurization.

Whitfield, Davis, and Downs (2) have shown that low-acid milk develops an oxidized flavor when contaminated with copper. At the West Virginia Station, when an incidental check on the effect of neutralization of acidity on the development of oxidized flavor was made, the results were at such variance with those of Anderson and his co-workers that the following experiments were conducted.

EXPERIMENTAL

The milk used in these trials was produced by the West Virginia Experiment Station herd, which consisted of a mixture of animals of the Jersey, Guernsey, Holstein, and Ayrshire breeds. All milk used in these trials was produced on dry feed. In the experiments with individual cows' milk, the milk was drawn into aluminum pails, and a sample, taken by means of a glass beaker, was transferred directly to a glass bottle. After all the samples were collected they were brought immediately to the laboratory, where the acidity was determined by titrating 17.5 ml. of milk with N/10 NaOH by use of 3 to 5 drops of phenolphthalein as an indicator; the results were

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[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 207.

[2] Department of Dairy Husbandry.

[3] Department of Agricultural Chemistry.

expressed in terms of per cent lactic acid. At no time did more than two hours elapse between the time the samples were drawn and the time the acidity determinations were made.

Following the determination of acidity all the samples were pasteurized by heating in the glass bottles to 144° F. ± 1° for 30 minutes followed by cooling to 60° F.

After cooling, each sample was divided into two parts; one part served as a control while the other was contaminated with 1.3 p.p.m. of copper added from a copper sulphate solution, this procedure having already been shown to develop oxidized flavor in susceptible milk. Both samples were then placed in ice water and allowed to remain there for three days, when they were removed and scored for flavor by at least three judges familiar with the off-flavor. All scoring was done without knowledge of the acidity of the milk as originally drawn.

The results of a study of 220 individual cows' samples are shown in Figure 1. An examination of the "line of least squares" shows that no relation exists between the acidity of freshly-drawn milk and the tendency to develop oxidized flavor. Both normal and oxidized-flavored milk was found in the low- and the high-acid milks in roughly the same proportion.

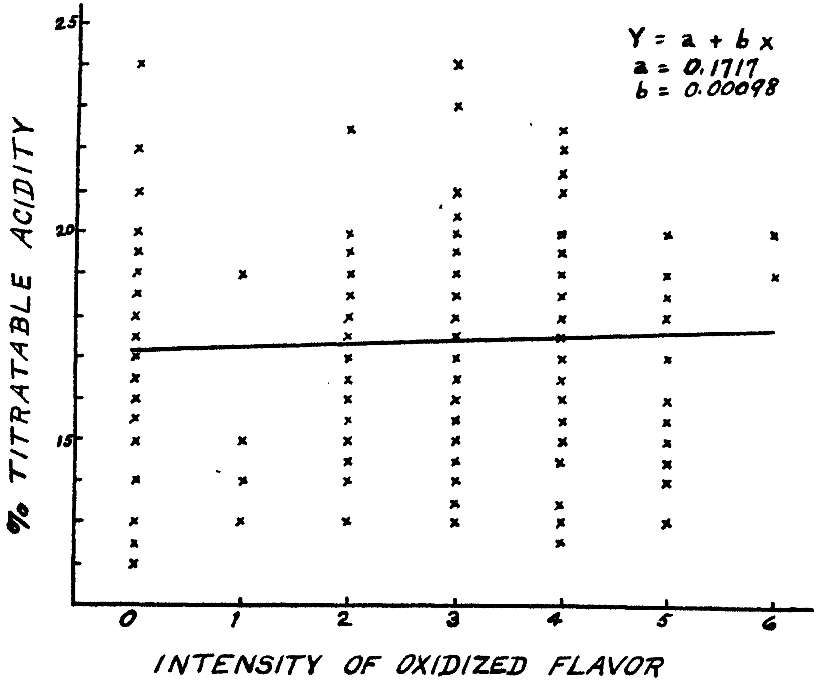

FIG. 1. The relation between acidity and metal-developed oxidized flavor in the milk of individual cows. (Each cross represents from 1 to 8 determinations.)

In the determination of oxidized flavor in individual cows' samples, three cases of spontaneously developed oxidized flavor occurred.

From the results, shown in Table 1, it would appear that there is a negative correlation between the acidity and the intensity of oxidized flavor, but because of the small number of observations no conclusions can be drawn.

TABLE 1

Relationship between acidity and spontaneous oxidized flavor development

Cow No.	Titratable acidity	Intensity of oxidized flavor*
	Per cent	
524	0.205	1
347	0.190	2
529	0.150	3

* For meaning of symbols see Table 2, footnote.

Since there was apparently no correlation between the acidity of freshly-drawn milk and the intensity of metal-developed oxidized flavor, it seemed advisable to determine whether or not neutralization had any effect on the tendency to develop the flavor.

STANDARDIZATION OF ACIDITY

In the standardization of acidity, the acidity of the milk was determined after the raw milk had been placed in the vat. A concentrated solution of neutralizer was used to neutralize the natural acidity of the milk. The neu-

TABLE 2

The development of oxidized flavor in unneutralized milk

Date	Source of sample			
	Raw milk	Pasteurized milk		
	In vat	In vat	End of the line—first over equipment	End of the line—taken when vat ½ full
	Intensity of oxidized flavor*			
2/17/38	3	4	4	4
2/21/38	3	4	4	4
2/25/38	3	4	4	4
3/ 1/38	3	3	3	missed
3/ 6/38	3	4	3	3
3/12/38	3	4	4	3
3/19/38	3	3	3	3
3/20/38	2	4	4	4
3/26/38	4	4	4	4

* Flavor developed by the addition of 1.3 p.p.m. copper. Control samples all free from oxidized flavor.

Meaning of symbols: –, no oxidized flavor; 1, very slight oxidized flavor; 2, slight oxidized flavor; 3, moderate oxidized flavor; 4, fairly pronounced oxidized flavor; 5, pronounced oxidized flavor; 6, very pronounced oxidized flavor.

tralizer was added, just before pasteurization, when the milk was at a temperature of from 80 to 90° F. The milk was held at this temperature for 10 to 15 minutes. The final acidity was checked in the milk after it had passed over the equipment. "Neutralene," C.P., Na_2CO_3, and $NaHCO_3$ were used to reduce the acidity. No difference in flavor was found due to differences in neutralizers.

The intensities of the oxidized flavor developed in unneutralized milk are given in Table 2. This flavor was developed by the addition of 1.3 p.p.m. of copper added from a copper sulphate solution followed by storage for three days in ice water. The control samples, to which no copper was added, did not develop an oxidized flavor, and these results were omitted from the table. The acidity was not determined on the samples reported in Table 2, but as all this milk was from one source, the acidity probably varied between 0.16 and 0.18 per cent. All samples that were contaminated with copper developed an oxidized flavor. As was to be expected from previous experimental work, the pasteurized milk developed a slightly stronger oxidized flavor.

The effects of neutralization on the intensity of oxidized-flavor development are shown in Table 3.

TABLE 3

The development of oxidized flavor in neutralized milk

Date	Per cent acid		Source of sample			
			Raw milk	Pasteurized milk		
	Original	Final	In vat	In vat	End of line—first over equipment	End of line—taken when vat ½ full
			Intensity of oxidized flavor*			
3/14/38	0.170	0.150	3	4	3	3
3/15/38	0.165	0.130	3	4	4	3
3/16/38	0.165	0.140	3	4	3	3
3/17/38	0.165	0.140	3	4	4	4
3/18/38	0.165	0.140	3	4	4	4
3/21/38	0.165	0.145	4	4	4	3
3/22/38	0.175	0.140	4	4	4	3
3/23/38	0.175	0.100	4	4	4	3
3/24/38	0.180	0.110	4	4	3	3
3/25/38	0.175	0.130	4	4	4	missed
3/27/38	0.175	0.095	4	4	4	3
3/28/38	0.175	0.135	4	4	4	missed
3/29/38	0.170	0.125	4	4	4	4
3/30/38	0.175	0.125	4	5	4	missed
3/31/38	0.180	0.125	3	4	4	4
4/ 1/38	0.170	0.160	4	4	4	4
4/ 2/38	0.180	0.140	4	4	4	3

Samples 3/14 to 3/23/38—neutralized with neutralene.
3/24 to 3/29/38—neutralized with Na_2CO_3.
3/30 to 3/31/38—neutralized with neutralene.
4/ 1 to 4/ 2/38—neutralized with $NaHCO_3$.

* For meaning of symbols see Table 2, footnote.

Analysis of these results shows that standardization of the acidity of milk to 0.13 per cent did not affect the tendency for the milk to develop oxidized flavor when that milk was contaminated with copper. In the two cases in which the milk was neutralized to 0.10 and 0.095 per cent, respectively, the tendency for oxidized flavor to develop was unchanged. In all cases in which the neutralization carried the acidity below 0.13 per cent there was a noticeable neutralizer flavor, but the oxidized flavor could easily be noticed above this. These results show definitely that neutralization does not affect the tendency for oxidized flavor to develop.

DISCUSSION OF RESULTS

No explanation is offered for the discrepancy in results which have occurred except that possibly the milk used by Anderson and his co-workers was subject to the development of oxidized flavor without copper contamination while the milk used in these trials would not develop oxidized flavor unless contaminated with copper. In all trials herein reported the copper was added after pasteurization in the form of a copper sulphate solution. In view of this fact it would have been desirable to make several trials upon naturally susceptible milk, but during the past two years milk of this type has not been available, except in rare cases, in the University herd.

In the few cases in which oxidized flavor developed spontaneously no direct relation was apparent between acidity and the intensity of the oxidized flavor developed.

Probably the chief reason for the belief that oxidized flavor might be related to the acidity is the fact that summer milk, which is usually non-susceptible to oxidized flavor, is generally lower in acidity than is winter milk which, when produced on dry feed, is usually susceptible to oxidized flavor if contaminated with copper.

CONCLUSIONS

1. There does not appear to be any relationship between the acidity of freshly-drawn milk and the intensity of metal-developed oxidized flavor.

2. Standardization of the acidity of fresh milk to 0.13 per cent did not prevent the development of oxidized flavor when copper is added after pasteurization.

ACKNOWLEDGMENT

The authors wish to express their appreciation for the assistance of Mr. L. J. Manus, Instructor in Dairy Husbandry, and Mr. L. V. Menefee, University creamery superintendent, in determining the flavor of experimental samples.

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A RAPID METHOD FOR DETERMINING MOISTURE IN ROUGHAGES

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In operations where moisture control is necessary it is of prime importance to be able to determine the moisture content of the material quickly. The usual oven-drying methods which take from 12 to 24 hours are too slow to be of great help in such cases. A rapid yet reasonably accurate method of determining moisture can be of the greatest value in establishing experimentally the conditions most desirable for storing hay and silages of various kinds. Such a method would likewise seem to offer great possibilities in the control of these operations under practical farm conditions, thus assuring uniformly better results.

While seeking for a quick, simple method for determining moisture in roughages it was discovered that there was on the market an apparatus[1] designed for the rapid determination of moisture in controlling certain manufacturing processes. This seemed to offer possibilities for use in drying grass and hay samples. The rapid removal of moisture is accomplished in this machine by forcing heated air through the sample. Essentially the apparatus consists of an electric fan and heating element encased in a tube which is enlarged at the base. A removable pan containing the sample to be dried is held snugly against the base by means of a spring clamp. The bottoms of the sample pans are specially constructed of 500-mesh screening to permit the passage of air. The complete apparatus including the necessary balances, drier, and utensils is shown in the accompanying illustration (Fig. 1).

In order to check the accuracy of the results obtained by the machine-drying, some samples have been dried by this process and also by the usual oven method. In Table 1 some of the results of this comparison are shown. In the oven-drying process the samples were allowed to remain in an electric oven for approximately 24 hours; the temperature was regulated to 100° C. After removal from the oven the samples were cooled to room temperature in a desiccator before weighing.

For the machine-drying process the time of drying ranged from 10 to 30 minutes, as shown in the table. The finer samples of grass required less time than corn silage containing some coarser particles. It was found desirable to stir the sample gently at approximately the middle of the drying process. After the pans were removed from the drier they were allowed to cool to room temperature in the open before weighing. All samples were weighed on a

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[1] Manufactured and sold by Harry W. Dietert Co., 9330 Roselawn Avenue, Detroit, Michigan.

balance with a sensitivity of 20 milligrams. Green material dried in this apparatus retained its original color remarkably well, although the temperature of the air forced through the material ranged from 105 to 115° C.

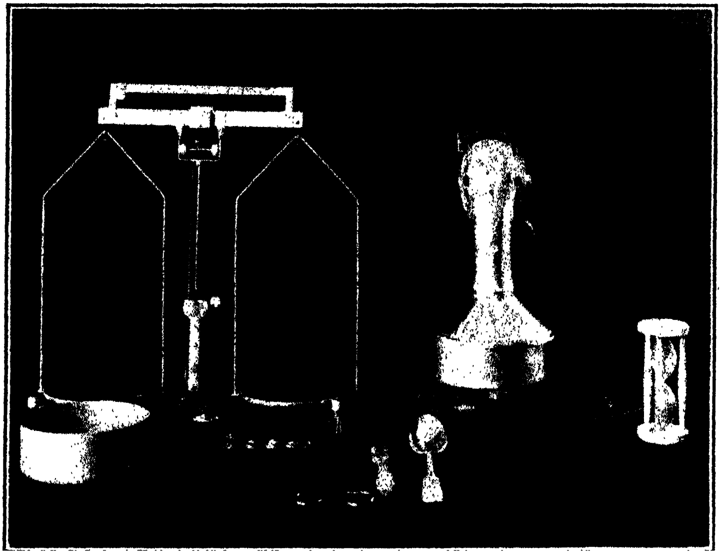

FIG. 1. Apparatus used in the rapid determination of moisture in roughages. Left rear—Balances, sensitivity 20 milligrams. Right rear—Drying machine with sample pan in position. Foreground—(left to right) Sample pan, weights, tongs, brush, spoon, and 10-minute time-glass.

TABLE 1

Comparison of results of dry-matter determinations by machine-drying and by the usual oven method

(Determinations made in triplicate)

Sample	Weight of wet sample	Time of drying in machine	Dry matter: Machine dried	Dry matter: Oven dried
	Grams	*Minutes*	*Per cent*	*Per cent*
Corn silage (whole)	20	30	26.35	25.9
Corn silage (whole)	20	30	25.2	25.4
Corn silage (whole)	50	30	29.3	28.1
Average			26.95	26.47
Green rye	20	10	15.3	15.0
Bluegrass	20	10	24.5	24.0
Bluegrass	20	10	27.0	26.75
Bluegrass	20	10	21.7	20.41
Average			24.4	23.72
Red clover	20	15	23.7	24.2
Alfalfa hay	20	10	89.5	89.5

In most cases 20-gram samples were used, although it is possible to use somewhat larger samples for some materials. The time required for drying did not seem to be so much influenced by the size of the sample as by the coarseness of the material. The materials used were cut into lengths ranging from 1 to 2 inches for convenience in sampling and weighing. Corn silage was sampled without further chopping. The thick stalks in the silage necessitated a longer drying period.

A fairly close agreement was found between the results obtained by the two methods. When proper consideration is given to the possibility of sampling errors, the small samples used, and the rather crude system of weighing, the agreement is probably all that could be expected. For most control work the results obtained by the machine drier are sufficiently accurate to justify its use. In fact, with additional refinements this process of machine drying may even be suitable for some kinds of laboratory work. The samples dried in the machine showed less discoloration, an evidence of deterioration, than did those dried in the oven for 24 hours at 100° C.

SUMMARY

Moisture was determined in the same samples of roughage by, 1, drying in an electric oven for 24 hours at 100° C.; 2, drying 10 to 30 minutes in a machine designed to force heated air through the material. The results obtained by the two methods showed a satisfactory agreement.

THE CALCULATION OF FAT CORRECTED MILK BY MEANS OF A NOMOGRAPH CHART

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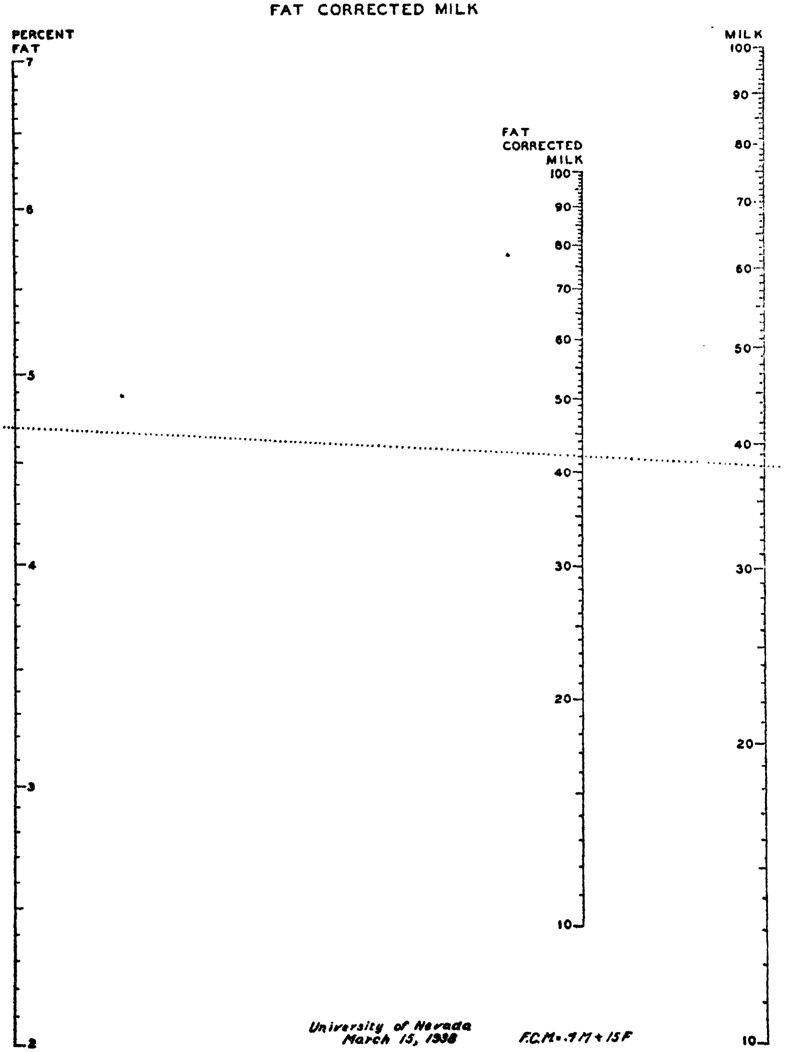
It has recently been pointed out by A. E. Perkins[1] that the reduction of weights of milk of varying fat content to a uniform fat-corrected basis has a



Received for publication June 13, 1938.

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[1] Perkins, A. E. A Simplified Procedure for Calculating Weights of Milk to Their Energy Equivalent in Milk of Different Fat Content in Accordance with the Gaines Formula. JOUR. DAIRY SCI. 20: 129–132. 1937.

number of advantages. It is, therefore, not necessary to discuss again the uses which may be made of this procedure.

Gaines formula for making this correction is as follows:

$$FCM = 0.4M + 15F$$

in which FCM = weight of fat corrected milk, M = weight of milk and F = weight of fat. The weight of the fat is obtained by multiplying the weight of the milk by the percentage of fat.

Professor Perkins has shown that the time required to make calculations may be considerably shortened by multiplying the pounds of milk by a factor which is dependent on the percentage of fat in the milk.

The problem may also be quickly solved without any mathematical computation by using the accompanying nomograph chart. Problems are solved by laying a ruler, or other straight edge, from the pounds of milk in the right-hand scale to the percentage of fat in the left-hand scale and reading the answer in the center scale.

For example: Find the FCM in 38 pounds of milk testing 4.7 per cent fat.

Solution: Lay a straight edge from 38 in the right-hand scale to 47 in the left-hand scale and read the answer 42 pounds, in the center scale, as indicated by the dotted line.

THE RELATION OF THE QUALITY OF MILK TO THE GRADE OF SWISS CHEESE[1]

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While it is generally conceded that good milk, from a bacteriological standpoint, is essential for making a high grade cheese of any variety, and particularly of the Swiss type, there is little definite information to support this contention. Donner and Ritter (1), compiling records on 42 cheeses made in Switzerland, found that the proportion of first grade cheese was at its maximum when the milk reduced methylene blue in 3 to 4 hours. There was a sharp decrease in the proportion of first grade cheese when the reduction time was less than 3 hours.

During the past 2 years the Bureau's portable field laboratories, operating in the Swiss-cheese district of Ohio in cooperation with the Ohio State University and in Wisconsin in cooperation with the University of Wisconsin, have accumulated records that warrant drawing certain conclusions. Since the cheeses on which these records were obtained were the regular factory product, without the checks and controls essential in properly conducted experimental manufacture, it is necessary to consider the data statistically. Obviously, it is safe to draw conclusions from statistical evidence only if the volume of data is sufficient and the sampling is representative. The data under consideration was obtained from slightly more than 500 cheeses made in a period of 2 years in 20 factories in Wisconsin and Ohio. While the number of cheeses in some of the groups is too small to justify any but tentative conclusions, the trends in the large groups are definite and consistent. There is some basis for criticism in the fact that these laboratories worked more with the factories having manufacturing difficulties than with those normally making good cheese. However, the factories from which these data were obtained are fairly representative and include some of the best in each state.

The quality of the milk was determined by a methylene blue reduction test made by a standardized method on the kettle milk. Samples were taken before adding starters or rennet.

In tabulating the quality of the cheese the grades established by the buyers

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[1] Since this paper was written an abstract of a paper on this subject has appeared: Erekson, A. B., Eckburg, C. A., and Lee, E. Methylene blue reduction time as an indication of the suitability of milk for the manufacture of Swiss cheese. JOUR. DAIRY SCI. 21: 172, 1938. The conclusions, based on records of 2,463 cheeses, are in substantial agreement with those presented in this paper.

TABLE 1

Relation of methylene blue reduction time to grade of cheese
Wisconsin and Ohio cheese 1936–1937

Reduction time	Total No.	A		B		C		D		A + B		C + D	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Not over 2 hrs.	80	12	15.00	17	21.25	30	37.50	21	26.25	29	36.25	51	63.75
Over 2 hrs., not over 3 hrs.	112	10	8.93	22	19.64	51	45.54	29	25.89	32	28.57	80	71.43
Over 3 hrs., not over 4 hrs.	119	18	15.13	49	41.18	40	33.61	12	10.08	67	56.30	52	43.70
Over 4 hrs., not over 5 hrs.	138	30	21.74	63	45.65	30	21.74	15	10.87	93	67.39	45	32.61
Over 5 hrs.	56	21	37.50	18	32.14	8	14.29	9	16.07	39	69.64	17	30.36

were used. It is recognized that this is by no means an exact measure of cheese quality, but it is the criterion on which the cheeses were sold and is the only one available.

In these tabulations the cheese grades are designated as A, B, C, and D, corresponding to the usual commercial grades. In Ohio these grades are known as No. 1, Special, No. 2, and Grinder; in Wisconsin the corresponding grades are designated as Fancy A, Fancy B, Fancy C, and Grinder. C and D may be considered as undergrades.

Table 1 shows the relationship between the reduction time and the percentage of cheese in each grade. While the correlation between the methylene blue reduction time and the grade is not entirely consistent there is a very distinct tendency for the grade to become better as the reduction time increases. This table also indicates that there is a distinct increase in the proportion of the two better grades when the reduction time passes 3 hours. This is shown more strikingly in Figure 1, in which the cheeses are divided into two groups, one including all cheeses made from milk in which the reduction time was not over 3 hours and the other, including all cheeses made from milk which required more than 3 hours for reduction. All cheeses in the A and B grades are combined in column 1 and those in the undergrades C and D in column 2. When the reduction time was 3 hours or less there were more than twice as many cheeses in the undergrades as in the A and B grades; when the reduction time was over 3 hours the results were reversed, with nearly twice as many in the A and B grades as in the undergrades.

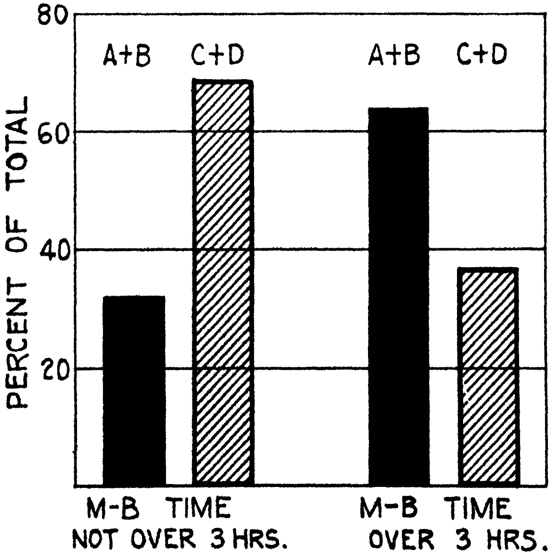

FIG. 1. The relation of methylene blue reduction time to the grade of the cheese.

While it is very evident that there is a direct relation between the quality of the milk as indicated by the methylene blue reduction time and the grade of the cheese, the bacteriological reason for this correlation is not so clear. Frazier (2) has shown that when bacteria of the aerogenes type are present in large numbers they multiply in the kettle and even if they are completely suppressed by the lactic bacteria they have a distinctly unfavorable effect on the quality of the cheese. It has also been observed that some of the spore-forming bacteria occurring in poor milk may develop in the cheese during the ripening and cause off-flavors and abnormal eye formation.

These conditions may occur when the bacteriological condition of the milk is unsatisfactory and they may account for at least a part of the inferior quality of the cheese.

There is also evidence of a correlation between the bacteriological condition of the milk and the rate of acid development during the making process. Under what may be termed normal conditions there is a small increase in the acidity of the milk in the kettle, represented by a drop in pH from about 6.6 in the fresh milk to from 6.5 to 6.4 at dipping. If the milk is well inoculated with acid forming bacteria and these are reinforced by an active starter, the pH change is more rapid and to a lower level.

The relation of this difference in acid development to the quality of the cheese is shown in Figure 2. The first four columns show the relative proportion of first and second grade cheese made from milk that reduced methylene blue in 3 hours or less. The solid columns represent A and B grades and the undergrades are shown by hatched columns. There was practically no difference in the proportion of first and second grade cheese when the pH at dipping was below 6.36 and when it was between 6.36 and 6.51. In other

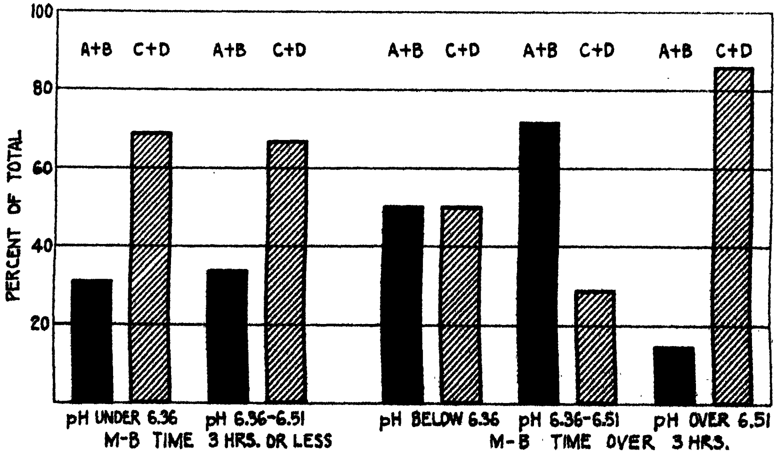

FIG. 2. Relation of hydrogen-ion concentration at dipping time to the grade of the cheese.

words, if the milk is so poor that it reduces methylene blue in 3 hours or less, the chances are about one in three that the cheese will be first grade and this ratio is not likely to be changed appreciably by differences in the rate of acid formation in the kettle.

The 6 columns on the right show the distribution of grades of cheese made from milk reducing methylene blue in more than 3 hours.

If the cheese is made from milk that has a reduction time greater than 3 hours and the pH at dipping is less than 6.36, the chances are even that the cheese will be in the A or B grade. If, however, the pH at dipping is between 6.36 and 6.51 the chances are three in four that the cheese will be A or B grade. If the bacteriological conditions in the milk are such that there is very little development of acid in the kettle and the pH at dipping is above 6.51, it is highly probable that the cheese will be undergrade. These results indicate that the chances of obtaining an A or B grade cheese are only a little better than 1 in 10, but this is based on a small number of cheeses and is only tentative. However, it confirms the generally accepted belief of the cheesemakers that milk which works slowly, so-called "dead" milk, is not suitable for Swiss-cheese making.

CONCLUSIONS

One of the most important factors influencing the quality of Swiss cheese is the bacteriological condition of the milk. This can be determined satisfactorily for manufacturing purposes by the methylene blue reduction test. If the methylene blue reduction time on the kettle milk is not over 3 hours the chances are about one in three that the resulting cheese will be of the A or B (No. I or Special) grade. If the reduction time is over 3 hours, 2 out of 3 cheeses may be expected to be of the A or B grade.

The chances of making an A or B grade cheese may be increased to three out of four, by adjusting the starters and making process to secure a pH at dipping above 6.35 and not over 6.51. If the pH at this time is over 6.51 there is only 1 chance in 8 or 10 that the cheese will be of the A or B grade.

It does not necessarily follow that the rapid increase in acidity indicated by a pH of 6.35 or lower at dipping time is in itself responsible for the inferior quality of the cheese which frequently results when these conditions exist. The conditions which produce a rapid development of acid may also promote the growth of abnormal bacteria having an unfavorable effect on the curd. Some of the increase in acidity may be due to ordinary lactic streptococci, which have an opportunity to grow for a short time in the early stages of the making process, and when the bacterial content of the milk is high other bacteria may develop until they are checked by the high temperature of cooking. It has been pointed out that certain of these bacteria may have a deleterious effect even though their activity in the kettle is limited.

It is highly probable, however, that when the pH at dipping goes as low as 6.30 or even 6.20, as it frequently does under factory conditions, it is due to an active thermophilic coccus which develops even during the cooking process and that the defective cheese may be attributed directly to the abnormally high acidity.

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LIVE WEIGHT AND MILK-ENERGY YIELD IN THE WISCONSIN DAIRY COW COMPETITION

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The Wisconsin Dairy Cow Competition was conducted during the years 1909–1911 and the records were published in some detail by Woll (1). Brody and Cunningham (2) recently utilized Woll's data to show that gross efficiency is independent of live weight. The object of the present paper is to extend their observaton to show that, in these records, milk-energy yield per unit live weight is independent of live weight. As will be developed, these two relations permit the deduction that the digestible feed energy of working maintenance is proportional to live weight (rather than a fractional power of live weight). The utilization of live weight and milk-energy yield per unit live weight in a philosophy of dairy cattle breeding will be mentioned.

LIVE WEIGHT AND GROSS EFFICIENCY

Gross efficiency is defined as the percentage ratio of milk-energy yield to digestible feed energy consumed. What is the relation of this ratio to live weight as shown by Woll's data?

Milk-energy yield is estimated from the reported milk and fat yields in terms of pounds of 4-per-cent milk (FCM) by the .4M + 15F formula. Feed consumption is reported by Woll in terms of feed units (FU), one FU being the equivalent of one pound of corn. Live weight in pounds (W) is given for 369 cows. FCM, FCM/FU and FCM/W have been computed for each of the 369 cows. For present purposes we are not concerned with the absolute values of gross efficiency but only its relation to live weight, which is equally well shown by FCM/FU.

The correlation surface for W and FCM/FU is shown graphically in Figure 1, and gives $r = +.345 \pm .032$. This result seems to contradict the statement that gross efficiency is independent of live weight. However, Woll allows 8 FU per day on pasture for each cow regardless of her live weight, although he states, "the number of units at which pasture should be rated will vary greatly according to its quality and the production and live weight of the cows and may range between 4 and 12 units per day." Qualitatively, correction of this rather serious error in the estimate of feed consumption would reduce, algebraically, the above value of r; quantitatively, it is quite possible it might reduce r to zero. We may accept provisionally the conclusion of Brody and Cunningham that, in these records, gross efficiency is independent of live weight.

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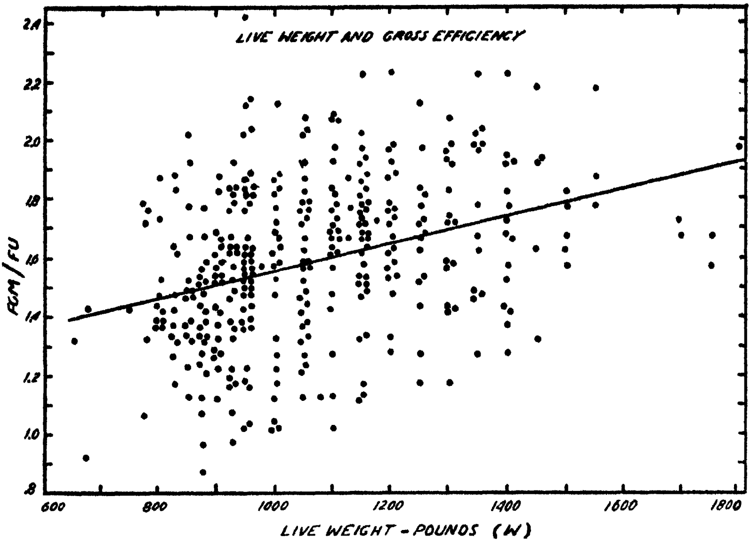

FIG. 1. Relation between milk-energy yield in pounds of 4-per-cent milk per feed unit consumed (FCM/FU) and live weight in pounds (W). The regression, shown by the straight line, is FCM/FU = 1.096 + .000465W. Gross efficiency (100 × milk calories/ digestible feed calories) = 23.73 (FCM/FU), computed as milk calories = 340FCM and digestible feed calories = 1433FU.

LIVE WEIGHT AND MILK-ENERGY YIELD PER UNIT LIVE WEIGHT

The correlation surface for W and FCM/W is shown graphically in Figure 2, and gives r = – .023 ± .035. It is clear that live weight and milk-

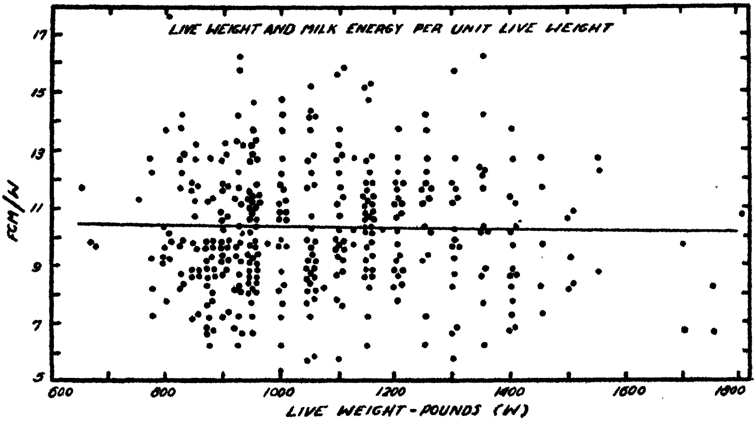

FIG. 2. Relation between milk-energy yield in pounds of 4-per-cent milk per pound live weight (FCM/W) and live weight in pounds (W). The regression, shown by the straight line, is FCM/W = 10.531 – .000256W.

energy yield per unit live weight fluctuate independently of each other. Or, milk-energy yield tends to be a multiple of live weight.

LIVE WEIGHT AND WORKING MAINTENANCE

We may split feed consumed, FU, into two parts: feed for lactation, FU′; and feed for working maintenance, FU″; such that FU = FU′ + FU″. We may assume FU′ is proportional to FCM, and hence proportional to W, in these records. If FCM/FU is constant so far as related to W (gross efficiency independent of live weight) and FCM is proportional to W, it follows that FU is proportional to W, and FU″, the feed for working maintenance, must also be proportional to W.

While it is recognized the reported feed consumption of individual cows is erroneous with respect to pasture, this would not warrant the deductions made except for the support of other findings (3, 4).

DISCUSSION

Under rules of the Competition entry was open to cows of any breed and records might start with the fifth or any later day after calving and continue not to exceed 365 days during the same lactation. Grade and registered cows of the Jersey, Guernsey, and Holstein breeds were entered. The latest stage of lactation of any entry was 637 days after calving (No. 322, days in milk = 314, FCM = 9999.9). The shortest time in milk was 269 days (No. 295). It is obvious that such variation in days in milk and stage of lactation must have an effect on both yield and gross efficiency. In Table 1 correlations are given for subgroups by days in milk and by breed of cow.

It will be noted in Table 1 that while for all breeds together W and FCM/W are independent, the same is not strictly true within any one of the breeds. This difference is associated with the fact that FCM/W is lowest for the smallest breed, and highest for the largest breed, thus:

Breed	365-day records		Other records	
	mean W	mean FCM/W	mean W	mean FCM/W
Jersey	930	10.125	903	8.899
Guernsey	997	10.514	974	9.534
Holstein	1272	11.346	1202	10.830

It is quite unexpected to find such a condition, and it may be that as a breed the Holsteins had more nearly optimum conditions for maximum production than did the Jerseys, with the Guernseys intermediate. However that may be, the results emphasize the necessity of taking size of cow into account in a breeding philosophy (*cf.* 5, 6).

TABLE 1

Coefficients of correlation (r) between live weight (W) and milk-energy yield per unit live weight (FCM/W), and between live weight (W) and milk-energy yield per feed unit (FCM/FU) by length of record and by breed of cow

Breed	Item	365-day records*	Other records	All records
Jersey	r, W and FCM/W	−.59	−.22	−.27
	r, W and FCM/FU	−.08	+.31	+.25
	number of cows	17	59	76
Guernsey	r, W and FCM/W	−.47	−.12	−.22
	r, W and FCM/FU	−.13	+.28	+.12
	number of cows	68	76	144
Holstein	r, W and FCM/W	−.23	−.64	−.45
	r, W and FCM/FU	+.20	+.15	+.22
	number of cows	67	82	149
All Breeds	r, W and FCM/W	−.06	−.06	−.02
	r, W and FCM/FU	+.31	+.32	+.35
	number of cows	152	217	369

* Records starting within 30 days after calving and continuing 365 days in milk. An important point, on the side, in the 365-day records of all breeds together is the relation of age to yield. Dealing with cows under 6 years of age (106 cows, ages 1.79 to 5.90 years) to approximate linear regression of yield on age, the correlation between age and FCM is, $r = +.30$, and $FCM = 8998 + 762 \times age$; between age and FCM/W, $r = +.14$ and $1000FCM/W = 9742 + 234 \times age$. Most of the age trend is removed in FCM/W and use of initial live weight for W would practically remove the age trend completely. That is, FCM/W needs no "age corrections."

DAIRY CATTLE BREEDING PHILOSOPHY

1. Breed for size of cow, that is, set up size as a first characteristic. We can breed cows of any size from say 700 pounds to 1500 pounds with some degree of certainty. Some dairymen need large cows, others need small cows. This need can be directly met.

2. Breed for composition of milk, that is, set up milk composition as a second characteristic. Fat percentage may be a satisfactory measure of composition. We can breed for milk of any fat percentage from say 3 to 6 with some degree of certainty. Some dairymen need cows giving milk of high fat percentage, others, low fat percentage. This need can be directly met.

3. Breed for hard-working cows, that is, set up degree of work in lactation as a third characteristic. Milk-energy yield per unit live weight may serve as a measure of degree of work. All dairymen need cows with high development in this regard. It is the most important to have, and least certain to breed for, of the three characteristics.

The point of view is that the inheritance of milk production in the dairy cow goes back to having an organism strong enough to do the work, accomplish the energy transformations, directly and indirectly connected with heavy long-time milk giving. Size of itself is thus a primary consideration. Knowing the size of the organism, the next consideration is to measure its proclivity to milk secretion, to obtain quantitative data for genetic study of this phase. Obviously, absolute milk yield is not directly suitable. Milk-energy yield per unit live weight may be directly suitable. Appropriate units need to be chosen and appropriate environment provided.

The above point of view attempts to distinguish between high yield associated with large size of cow and high yield at a given size of cow. If we breed larger cows and secure higher yields, it may be that we have progressed only in the first characteristic (size) and not at all in the most important third characteristic (degree of work, or proclivity to lactation). It is necessary to distinguish between these two phases of development in the dairy cow.

SUMMARY

Records of 369 cows (Jersey, Guernsey, Holstein--grade or registered) show that milk-energy yield per unit live weight is independent of live weight $(r = -.023)$. The same records show that gross efficiency (milk energy/feed energy) increases with live weight $(r = +.345)$. Elimination of recognized systematic errors in the feed record may reduce the latter correlation to zero, making gross efficiency, in fact, independent of live weight. Then, a permissible deduction is that the feed of working maintenance is proportional to live weight.

In study of the genetics of milk yield in dairy cows, distinction should be made as to size of the organism which may be measured by live weight, and proclivity to lactation which may be measured by milk-energy yield per unit live weight.

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REPORT OF THE STUDENTS NATIONAL JUDGING CONTESTS

THE STUDENTS NATIONAL CONTEST IN JUDGING DAIRY CATTLE

Twenty-six teams competed in the Collegiate Dairy Cattle Judging Contest held in connection with the National Dairy Show at Columbus, Ohio, October 8, 1938. The rules under which the contest was conducted were the same as those used in 1937. Fifteen rings of animals, three rings from each of the five breeds, were judged and oral reasons were graded on one ring of cows from each breed.

The official Judges for each breed were:

Ayrshires: Jack Nisbet, Fort Atkinson, Wisconsin.
Brown Swiss: Fordyce Ely, University of Kentucky, Lexington, Ky.
Jerseys: Frank Astroth, South St. Paul, Minn.
Guernseys: O. G. Schaefer, Graybar Bldg., New York, N. Y.
Holsteins: L. S. Gillette, Fostoria, Iowa.

The results of the contest were announced at an American Dairy Science Association banquet in Pomerene Hall on the Ohio State University campus.

Teams from the following colleges and universities were represented and are listed in the order of their rank in judging all breeds.

1. Ontario Agricultural College
2. Iowa State College
3. University of Minnesota
4. South Dakota State College
5. Ohio State University
6. University of Missouri
7. Pennsylvania State College
8. University of New Hampshire
9. Purdue University
10. Texas Technological College
11. University of Tennessee
12. University of Wisconsin
13. University of Illinois
14. University of Arizona
15. Texas A. & M. College
16. Cornell University
17. Kansas State College
18. University of Georgia
19. Michigan State College
20. New Mexico College of A. & M.
21. Mississippi State College
22. Oklahoma A. & M. College
23. Rutgers University
24. Virginia Polytechnic Institute
25. Connecticut State College
26. University of Nebraska

FIVE HIGH RANKING INDIVIDUALS IN JUDGING ALL BREEDS

1st.	Vincent Kelly, Iowa State College
2nd.	P. Archibald, Ontario Agricultural College
3rd.	Ira Crawford, University of Missouri
4th.	L. H. Schultz, University of Wisconsin
5th.	Merrill Siglin, South Dakota State College

DONORS OF AWARDS FOR PROFICIENCY IN JUDGING WERE:

American Jersey Cattle Club.

$400.00 Research Scholarship, awarded to R. F. Schroeder, University of Wisconsin, High ranking individual in judging Jersey cattle. Also a plaque awarded to R. F. Schroeder.

Rotating Jersey Model, awarded to the Ontario Agricultural College, high ranking team in judging Jerseys. Also plaques to each member, coach and alternate of the Ontario team.

Ayrshire Breeders' Assn. of America.

A silver cup, awarded to C. F. McKenney, Virginia Polytechnic Institute, High ranking individual in judging Ayrshire cattle.
A rotating silver cup, awarded to the University of Georgia, high ranking team in judging Ayrshires. Also a silver plaque was awarded to the Georgia team.
A silver cup was awarded to each member and alternate of the Georgia team.

Brown Swiss Breeders' Assn.

An imported Brown Swiss Cow Bell, awarded to Stewart Sewell, Texas Technological College, high ranking individual in judging Brown Swiss cattle.
Pen desk sets, awarded to each member, alternate, and coach of the Purdue University team, high ranking team in judging Brown Swiss cattle.

American Guernsey Cattle Club.

Guernsey silver milk jug and sugar bowl, awarded to Joe Baxter, University of Tennessee, high ranking individual in judging Guernsey cattle.
A rotating Large Milk Can, awarded to the University of Minnesota, high ranking team in judging Guernseys. Also, a Guernsey silver milk jug and sugar bowl was awarded to each member, coach, and alternate of the Minnesota team.

Holstein-Friesian Assn. of America.

Gold Medal, awarded to R. Bagg, Ontario Agricultural College, high ranking individual in judging Holstein cattle.
Rotating cup, awarded to the Ontario Agricultural College, high ranking team in judging Holsteins.
Gold Medals, awarded to each member and alternate of the Ontario team.
Brief case, awarded to G. E. Raithby, coach of the Ontario team.

National Dairy Show.

Gold Medal, awarded to Vincent Kelly, Iowa State College, high ranking individual in judging All Breeds.
Silver Medal, awarded to P. Archibald, Ontario Agricultural College, second high ranking individual in judging All Breeds.
Bronze Medal, awarded to Ira Crawford, University of Missouri, third high ranking individual in judging All Breeds.
Rotating cup, awarded to the Ontario Agricultural College, high ranking team in judging All Breeds.

Successful Farming.

Successful Farming Canes to the three high ranking men in judging All Breeds (Vincent Kelly, 1st; P. Archibald, 2nd; and Ira Crawford, 3rd.)

J. B. Ford Company.

Gold watch, awarded to Vincent Kelly, high ranking individual judging All Breeds.
Rotating Glory Trophy, awarded to Iowa State College, 2nd ranking team in judging All Breeds.

Country Gentleman.

Zipper Bag, awarded to Vincent Kelly, high ranking individual judging All Breeds.

Starline, Inc.

Radio, awarded P. Archibald, Ontario Agricultural College, second high ranking individual judging All Breeds.

Hoard's Dairyman.

Rotating cup, awarded Ontario Agricultural College, high ranking team judging All Breeds.

THE STUDENTS' NATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Cornell University won the sweepstakes cup and a scholarship at the Students' Contest in Judging Dairy Products at the Dairy Industries Exposition in Cleveland on October 17. Iowa State College won second place, and Connecticut State College third. Both these colleges were also awarded scholarships. Teams from 23 colleges competed—the greatest number ever to compete in this contest.

Highest individual honors in judging all 4 products were won by L. M. McCalla, of Mississippi State College, who was awarded a gold medal. Silver and bronze medals for second and third places were awarded to Thomas S. Warner, of Iowa State College, and Nathan Andursky, of Cornell University, respectively.

Cornell University was also awarded 2 silver cups for placing first in judging ice cream and cheese. Iowa State College won the silver cup for placing first in judging butter, and Ohio State University received a similar award in the judging of milk.

Gold, silver, and bronze medals were awarded to the individual contestants who won first, second, and third places, respectively, in the judging of each of the four products.

The scholarships, cups, and medals were provided by the Dairy and Ice Cream Machinery and Supplies Association, which Association has sponsored this contest for 9 successive years.

The International Association of Milk Dealers awarded a gold medal to Howard Kizer, University of Illinois, for placing first in judging milk, and the International Association of Ice Cream Manufacturers a gold medal to L. M. McCalla, Mississippi State College, for placing first in ice cream.

Twenty-three teams, representing 22 State Agricultural Colleges and one Provincial Agricultural College of Canada, participated in this, the ninth annual contest sponsored by the Dairy and Ice Cream Machinery and Supplies Association, Inc., and the American Dairy Science Association.

Following are the names of the colleges and individual contestants who won high standings in the contest:

ALL PRODUCTS

Teams		*Individuals*	
1—Cornell University	**177.45**	**1—L. M. McCalla, Mississippi State College**	**50.00**
2—Iowa State College	**178.00**	**2—Thomas S. Warner, Iowa State College**	**52.50**
3—Connecticut State College	**193.90**	**3—Nathan Andursky, Cornell University**	**55.20**
4—Mississippi State College	**198.75**	**4—Lealand G. Sherman, Iowa State College**	**57.25**
5—Michigan State College	**199.20**	**5—Varnum D. Ludington, Cornell University**	**57.75**

ALL PRODUCTS

Teams	
6—University of Tennessee	200.25
7—Ohio State University	203.75
8—University of Maryland	215.50
9—Massachusetts State College	218.00
10—University of Vermont	226.25

Individuals	
6—P. A. Turner, Jr., University of Tennessee	59.25
7—Wayne E. Smith, Ohio State University	59.50
8—Frank Kosikowsky, Connecticut State College	61.25
9—Robert Stephenson, Michigan State College	63.00
10—Bradley L. Gilbert, Michigan State College	64.25

ICE CREAM

Teams	
1—Cornell University	65.0
2—Massachusetts State College	65.75
3—Kansas State College	68.0
4—Iowa State College	69.75
5—University of Tennessee	70.0
6—Mississippi State College	73.5
7—Connecticut State College	76.0
8—West Virginia University	76.75
9—University of Nebraska	77.0
10—University of Maryland	77.75
10—Michigan State College	77.75

Individuals	
1—L. M. McCalla, Mississippi State College	18.0
2—Marvin Koger, New Mexico College of A. & M. A.	18.75
3—Charles Rodda, Jr., Massachusetts State College	19.00
4—P. A. Turner, Jr., University of Tennessee	19.00
5—Varnum D. Ludington, Cornell University	19.50
5—Thomas S. Warner, Iowa State College	19.5
5—Melvin Jensen, South Dakota State College	19.5
8—Floyd Mecham, Jr., University of Nebraska	20.0
8—Willis Faulkender, Kansas State College	20.0
8—S. H. Thomas, Ontario, Agric. College	20.0

BUTTER

Teams	
1—Iowa State College	30.0
2—Cornell University	34.0
2—University of Maryland	34.0
4—Connecticut State College	35.0
5—Ontario Agric. College	38.0
6—Michigan State College	39.0
6—Oklahoma A. & M. College	39.0
6—University of Tennessee	39.0
9—University of Wisconsin	39.5
9—University of Minnesota	39.5

Individuals	
1—Thomas S. Warner, Iowa State College	6.0
2—Clifton Martin, Jr., University of Maryland	7.0
3—Nathan Andursky, Cornell University	7.5
4—Lloyd C. Bowers, University of Maryland	8.0
5—William C. Kueffner, University of Minnesota	9.5
6—Jack L. Garvin, West Virginia University	10.0
7—William J. Nelsone, Connecticut State College	10.5
7—Frank Kosikowsky, Connecticut State College	10.5
7—George M. Fau[illegible]old, Pennsylvania State College	10.5
7—Arthur D. Kasler, Ohio State University	10.5
7—Varnum D. Ludington, Cornell University	10.5
7—John Anderson, West Virginia University	10.5
7—Donald W. Glover, Purdue University	10.5

MILK

Teams	
1—Ohio State University	37.25
2—Michigan State College	38.45
3—University of Vermont	39.25
4—New Mexico College of Agric. & Mech. Arts	39.50
5—Iowa State College	41.25
6—Connecticut State College	41.90
7—Massachusetts State College	45.75
8—Cornell University	46.95
9—University of Tennessee	49.25
10—West Virginia University	49.70

Individuals	
1—Howard Kizer, University of Illinois	7.75
2—Charles Rodda, Jr., Massachusetts State College	8.00
3—Wayne E. Smith, Ohio State University	8.00
4—Arthur D. Kasler, Ohio State University	8.25
5—Raymond Worrell, New Mexico College of A. & M. A.	10.25
6—Edwin M. Hankinson, Michigan State College	10.45
7—Robert E. Plumb, University of Vermont	10.50
8—Lealand G. Sherman, Iowa State College	10.75
9—Alva S. Baker, University of Maryland	11.25
10—Winfred Jacobsen, University of Nebraska	11.75

CHEESE

Teams	
1—Cornell University	31.5
2—Mississippi State College	32.0
3—Texas Technological College	32.5
4—Iowa State College	37.0
5—Connecticut State College	41.0
6—Ohio State University	41.5
7—University of Tennessee	42.0
8—Michigan State College	44.0
9—Pennsylvania State College	46.5
10—University of Vermont	51.5
10—University of Maryland	51.5

Individuals	
1—L. M. McCalla, Mississippi State College	7.5
2—Arch G. Lamb, Texas Technological College	9.0
3—Nathan Andursky, Cornell University	9.5
4—Lealand G. Sherman, Iowa State College	10.0
5—Wayne E. Smith, Ohio State University	10.5
5—Thomas S. Warner, Iowa State College	10.5
5—Clayburn Harrell, Texas Technological College	10.5
5—Frank Kosikowsky, Connecticut State College	10.5
9—Melvin W. Diehl, Pennsylvania State College	11.0
9—Varnum D. Ludington, Cornell University	11.0

AMERICAN DAIRY SCIENCE ASSOCIATION
PRESIDENT'S LETTER

TO THE MEMBERS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION:

As an officer of the Association I readily embrace this opportunity to report to you the progress of certain features of the Association program.

The members should be aware that the publication of the Journal constitutes the major activity of the Association. The financial transactions relating to the Journal are a significant part of the Association operations; for a great many members the Journal comprises the only liaison with the Association; subscriptions constitute a major source of revenue. Because of this situation, the secretary, the editor, the Journal management committee (O. F. Hunziker, A. A. Borland and R. B. Stoltz) and all the officers are alert to every possibility for increasing memberships and subscriptions.

During November a member in each state was requested to serve as a membership representative in his state. Three members in Canada were likewise so requested. All these men responded with a willingness that was most reassuring. Their efforts are proving fruitful. An especial urgency in respect to memberships arises this year because of the new by-laws which prescribe the $5.00 admission fee for new members.

One of the activities of the Association which has heretofore not received adequate recognition is that pertaining to the various committees and subcommittees appointed by the different sections. The Extension, Production and Manufactures Sections all have their committees. Some of these committees are encountering especially perplexing questions; the committee members are working diligently. It is hoped the membership at large will recognize the role these committees play and that every assistance to facilitate the committee activities will be offered.

The activities of some of the general association committees also deserve attention. A committee on Student Chapters was appointed by President Gregory last year. It consists of J. A. Nelson, E. V. Ellington and A. A. Borland. The committee is studying the questions and is making plans to submit its proposals at an early date. Doubtless a definite and acceptable policy with regard to Student Chapters can be adopted.

Harold Macy, H. A. Ruehe and F. B. Morrison were appointed by President Gregory as a committee to modify present rules or formulate new ones pertaining to the Borden Awards. The committee is making a thorough study of all aspects of this matter. In due time this report will be available for consideration. The Association members should be advised, however, that pending the adoption of changes in these rules the procedures as employed the past year will be observed.

Another important committee appointed by President Gregory is the committee on dairy curricula. The general committee consists of H. B. Ellenberger, H. P. Davis, M. Mortensen, C. E. Wylie, C. L. Roadhouse. The sub-committee on dairy manufactures consists of C. L. Roadhouse, A. D. Burke, J. H. Erb, C. D. Dahle, W. V. Price, R. W. Bell, H. F. Judkins. The sub-committee on dairy production consists of C. E. Wylie, J. W. Bartlett, I. R. Jones, C. N. Shepardson, T. E. Woodward, C. L. Clevenger.

This committee has been at work for more than a year. There is no disposition to effect any undue speed in the committee's deliberations but assurance has been given that a progress report would be forthcoming at the Summer meeting in June. Some Association members assert that the question of the curriculum is one of the most pressing ones confronting dairy departments today.

At the Summer meeting in 1938 action was taken which prescribed the appointment of a resolutions committee to assume certain duties as enumerated in the Journal for August 1938, page 519. The committee appointed is: C. Y. Cannon, D. R. Theophilus, L. S. Palmer, G. G. Gibson, and L. M. Thurston. The attention of the sectional chairmen and secretaries particularly is directed to this committee.

The chairman of the program committee for the Summer meeting in June 1939 is E. V. Ellington. The other members of the committee are automatically appointed by virtue of the fact they are section chairmen; viz. Manufactures, P. A. Downs; Production, H. W. Cave; Extension, S. J. Brownell. The advisory members are the program committee chairmen the past two years, namely, T. S. Sutton for 1938 and H. P. Davis for 1937.

This program committee is well along with its plans for the Summer meeting at Moscow, Idaho, and Pullman, Washington, the week of June 26, 1939. Various unique features of both the technical part of the program and the entertainment are to be offered the members. These features together with the setting for the meeting and the new experience through having our Association assemble for the first time in the Pacific Northwest promise an event of great profit and pleasure.

Yours very sincerely

EARL WEAVER, *President*
American Dairy Science Association

December 30, 1938

JOURNAL OF DAIRY SCIENCE

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DETERMINATION OF CAROTENE AND VITAMIN A IN MILK

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Spectrophotometric methods have been used extensively for the determination of carotene and vitamin A. As far as we are aware, however, the estimation of vitamin A in milk by measurement of the absorption at 328 mμ has only been accomplished by the use of butterfat as a starting material (1, 2). This procedure is inconvenient and laborious since large samples of milk are required and the cream must be separated and churned. Wilkie (3) has outlined a method for the estimation of carotene and vitamin A in evaporated milk based upon the Roese-Gottlieb (4) method for the determination of fat in milk. In our experience the proportion of solvents specified by Wilkie did not extract the carotenoids from whole milk, but by modifying the procedure somewhat satisfactory results have been obtained.

While the data were being prepared for publication a similar extraction procedure by Willstaedt and With (5) has appeared. These authors did not use spectrophotometric methods, however, but determined both carotene and vitamin A with the step-photometer. The reading for vitamin A was based on the color developed with antimony trichloride.

PROCEDURE

In a separatory funnel 100 cc. of milk are placed, the following reagents are added successively and the solution is shaken vigorously after each addition: 15 cc. concentrated NH_4OH, 100 cc. ethyl alcohol (aldehyde-free), 75 cc. ethyl ether (peroxide-free), and 25 cc. of Skelly solve B (petroleum ether b.p. 60–70°). After 20 minutes the lower layer is drawn off and is again extracted with a mixture of 25 cc. ethyl ether and 10 cc. Skelly solve B. The combined extracts are allowed to stand in the separatory funnel for 30 minutes. A considerable amount of suspended matter settles out and should be drawn off; otherwise the results will usually be too high.

The extract is transferred to a distillation flask equipped with a small separatory funnel and the flask is placed in a water bath. The solvents are

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removed under reduced pressure with the temperature of the bath no higher than 70° C. Care should be taken that the level of the liquid in the flask is not below that of the water in the surrounding bath and that the heating is not continued after the solvents have been removed. Immediately after the solvents have come off, 10 cc. of ethyl alcohol are run in from the separatory funnel before the vacuum is released.

The liquid is transferred with an additional 35 cc. ethyl alcohol to a flask connected to a reflux condenser with ground glass joints. Two and one-half grams of potassium hydroxide are added and the fat is saponified by refluxing in an atmosphere of nitrogen for 20 minutes. After saponification 100 cc. of water are added and the mixture is cooled to 4° C. The unsaponifiable matter is extracted with three portions of ether, 75, 25, and 25 cc. The ether extracts are combined in a separatory funnel and washed with four portions of cold water, 100, 100, 50, and 50 cc. The first two washings should be done by gently rotating the funnel; the last two by vigorous shaking. The water in the ether is then frozen out by placing the flask on crushed dry ice. The solution is filtered through cotton and the flask and cotton are washed with cold ether. The filtrate and washings are placed in a distilling flask and the ether is removed under reduced pressure.

Special care should also be taken in this distillation. The temperature of the water bath should not exceed 70° C. and the flask should be agitated continuously as the last traces of solvent evaporate. Just as the last of the ether boils off about 5 cc. of methyl alcohol (aldehyde-free) is run into the flask from the separatory funnel before the vacuum is released. If the residue is allowed to dry out before adding the methyl alcohol the resulting solution may be turbid and difficult to clarify. The solution is made up to 10 cc. with methyl alcohol and any precipitate present is removed by centrifuging. The carotene content of this solution is determined with the visual spectrophotometer and the vitamin A as described by Baumann and Steenbock (1).

RESULTS

The carotene and vitamin A contents of several milks were determined by the above procedure and compared with results obtained by analysis of the butterfat from the same milks. The butterfats were analyzed by the method of Semb, Baumann, and Steenbock (6). Table 1 summarizes the results obtained. For samples 1–5 the amount of filtered butterfat weighed out corresponded exactly to 100 cc. of milk. In samples 6D and 6E an additional amount of fat was added to the milk. The source of the fat varied as noted in the table. It was dissolved in the 100 cc. of ethyl alcohol used in the extraction. In one case, 6D, the added butterfat was obtained from the same batch of milk as was analyzed, so the carotene and vitamin A calculated on a butterfat basis should not have changed. The calculated carotene content was 2.8, found was 3.0; calculated vitamin A was 8.9, found was 8.8.

TABLE 1

Comparison of milk and butterfat as starting material for the determination of carotene and vitamin A

Number of sample	Description of sample	Material analyzed	Butterfat in sample	Carotene (based on butterfat)	Vitamin A (based on butterfat)
			gm.	γ/*gm.*	γ/*gm.*
1A	Mixed market	Milk	4.1	4.4	8.5
1B	" "	Butterfat	4.1	4.7	8.5
2A	Mixed market	Milk	3.7	3.9	9.6
2B	" "	Butterfat	3.7	3.7	9.6
3A	Guernsey	Milk	4.3	5.3	7.3
3B	"	Butterfat	4.3	5.2	7.3
4A	Certified mixed	Milk	4.3	6.9	7.8
4B	" "	Butterfat	4.3	6.3	7.9
5A	Mixed market	Milk	3.7	3.8	8.7
5B	" "	Butterfat	3.7	3.3	9.5
6A	Holstein	Butterfat	15.0	2.9	8.5
6B	"	Milk	3.4	2.5	9.3
6C	"	"	3.4	2.8	9.3
6D	"	" *	6.4	3.0	8.8
6E	"	" †	6.4	5.4	9.3
7A	Mixed market	Milk	4.5	8.3	9.6
7B	" "	"	4.5	8.1	9.6
7C	" "	Butterfat	15.0	8.2	9.5
7D	" "	"	4.5	8.6	9.6
7E	" "	"	3.0	8.6	9.6

* Plus 3 gm. butterfat prepared from milk No. 6B.
† Plus 3 gm. butterfat prepared from milk No. 7A.

The butterfat added to the milk in 6E came from a different milk and here again the recovery was good; carotene, calculated 5.4, found 5.4; vitamin A calculated 9.4, found 9.3.

The amount of butterfat used as starting material did not affect the final results. In samples 7C, D, and E, the amount of fat used varied from 3 to 15 grams, but the carotene figure varied only 0.4 of a microgram and the vitamin A only 0.1 microgram per gram of butterfat. These checks are as good as duplicate determinations.

Often it was impossible to assay the milk samples as soon as they were received. In order to see if storage might affect the determination runs

TABLE 2

Effect of storage and freezing of milk on carotene and vitamin A content

No. of analysis	Treatment of milk	Carotene (based on butterfat)	Vitamin A (based on butterfat)
		γ/*gm.*	γ/*gm.*
1	None	6.0	7.5
2	Stored two weeks at 12° C.	6.2	6.7
3	Three weeks at 12° C. then frozen for 10 days	6.2	7.4
4	Same as No. 3 then stored 14 days again at 12° C.	6.0	7.2

were made on a certified sample immediately after it was received and later after storage under various conditions and for various periods of time. It was allowed to stand in an ice box for two weeks before a second analysis was made. It was stored in an ice box for another week and was then kept frozen for 10 days before the third determination was made. It was again stored in an ice box for another two weeks before the fourth analysis was made. The results of these analyses are given in Table 2 and show that storage and freezing apparently did not affect either the carotene or the vitamin A content of the milk.

SUMMARY

A method has been developed for determination of carotene and vitamin A that can be applied directly to milk. The results check well with those obtained when butterfat is used as the starting material.

Milk can be stored or frozen for considerable periods without affecting the carotene or vitamin A content.

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PRODUCTION OF DIACETYL FROM CITRIC ACID IN BUTTER CULTURES[1]

E. A. PRILL[2] AND B. W. HAMMER

In the normal ripening of a butter culture the important fermentation products come from two main sources, lactose and citric acid. The former yields lactic acid while the latter yields a series of neutral compounds (diacetyl, acetylmethylcarbinol and 2,3-butylene glycol), together with acetic acid and carbon dioxide. Lactic acid ordinarily is produced in ample amounts unless there is some irregularity in the culture, such as the presence of an active bacteriophage, but the products formed from citric acid are often in such low concentrations that the culture lacks flavor and aroma, and does not have the desired effect when used in butter manufacture.

Supplementing the small percentage of citric acid normally in milk by addition of this acid to milk used for butter culture results in a conspicuous increase in the acetylmethylcarbinol plus diacetyl content (4, 5) and, in accordance with this, the flavor of the butter made with such culture is improved (2, 8).

Although diacetyl is a very important aroma substance of butter cultures and butter, in most of the work reported it was not determined separately because of inadequacy of the methods available for determining the small amounts present. The colorimetric method recently developed by Prill and Hammer (7) readily permits the determination of minute quantities of diacetyl. It was used in studies on the fermentation of citric acid in butter cultures with special reference to production of diacetyl. The results are reported herein.

ANALYTICAL METHODS

The diacetyl was determined colorimetrically (7) by converting it into dimethylglyoxime and then into the intensely colored ammono-ferrous dimethylglyoximate. In determining acetylmethylcarbinol plus diacetyl, the sample with ferric chloride added was distilled as in the usual procedure (5); a small aliquot of the distillate was taken, additional hydroxylamine acetate added to maintain the proper concentration of this reactant when diluted to 6 ml., and the procedure then continued in exactly the manner used for the diacetyl. Actually, the values recorded for acetylmethylcarbinol plus diacetyl are the amounts of diacetyl obtained after oxidation with ferric chloride. Various workers have noted that this oxidation is

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[2] The studies reported were largely financed through a grant from Chas. Pfizer and Co., Inc., which provided particularly for the services of Dr. Prill.

not absolutely quantitative and for this reason a correction for the slight difference in molecular weights is not warranted.

All the results on diacetyl and acetylmethylcarbinol plus diacetyl are expressed as parts per million (p.p.m.).[3]

GENERAL METHODS OF PREPARING BUTTER CULTURES

The butter cultures studied were prepared from well pasteurized skim or whole milk by inoculating with one of the butter cultures carried in the laboratory. In some cases citric acid (the monohydrate was regularly used) was added to the milk as an aqueous solution with vigorous agitation (to avoid excessive coagulation). Depending on the type of experiment, the milk was either divided into a number of portions and the portions ripened at 70° F. for about 16 hours, or an entire volume was ripened and any desired division then made.

Further treatments of the butter cultures are given under the different series of trials.

RESULTS

Data on the effects of various treatments on the diacetyl and the acetylmethylcarbinol plus diacetyl contents of butter cultures are presented in four series of trials. In each series the trials were repeated until the general changes occurring appeared to be well established; for each series representative data are reported rather than all the trials.

In discussing the results, changes in the acetylmethylcarbinol plus diacetyl contents are referred to simply as increases or decreases in acetylmethylcarbinol (*the* carbinol); this is sufficiently exact, especially when the diacetyl contents are known, because acetylmethylcarbinol makes up such a large percentage of the total acetylmethylcarbinol plus diacetyl.

Series 1. Effects of Varying Amounts of Citric Acid Used in the Preparation of Butter Culture on Ripened Cultures Held Cold

The cultures were prepared in flasks with addition of different amounts of citric acid (0.00 to 0.30 per cent) to the milk. After ripening, the cultures were held in the cooler (36° to 45° F.). At intervals (usually successive days) each of the flasks was shaken and a portion of the contents removed for analysis. Data for two trials are given in Figures 1 and 2.

When analysed on the first day (about 10 hours after the ripened cultures had been placed in the cooler), the cultures with added citric acid regularly had higher diacetyl contents than those without added citric acid.

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[3] In various publications from this laboratory the diacetyl and the acetylmethylcarbinol plus diacetyl values are expressed as milligrams of nickel dimethylglyoximate obtained from a 200 or a 400 g. sample. For comparison, such results can be converted to p.p.m. of the actual fermentation products by multiplying the value for a 200 g. sample by 3 and the value for a 400 g. sample by 1.5.

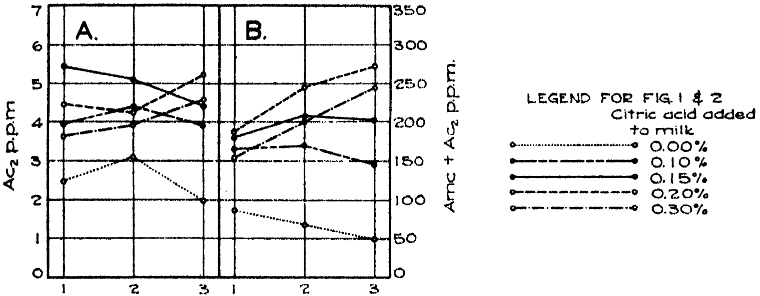

Figure 1

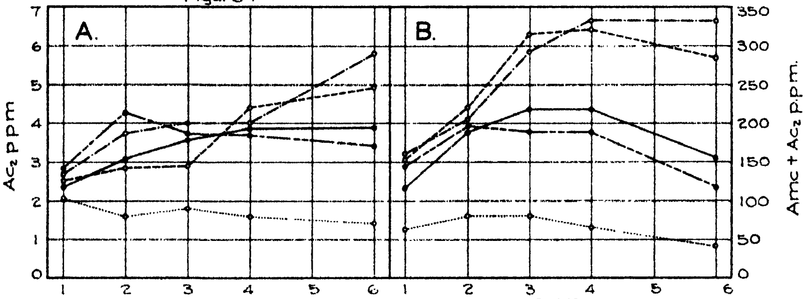

Figure 2

SERIES 1. EFFECTS OF VARYING AMOUNTS OF CITRIC ACID USED IN THE PREPARATION OF BUTTER CULTURES ON RIPENED CULTURES HELD COLD.—A. Values for diacetyl (Ac_2). B. Values for acetylmethylcarbinol plus diacetyl (Amc. + Ac_2).

The increases in diacetyl showed no relationship to the amounts of citric acid employed; in Figure 1 the diacetyl content was highest with 0.15 per cent added citric acid and next to the lowest with 0.30 per cent, while in Figure 2 there were only slight differences in the increases resulting from the various amounts of citric acid. During succeeding days the diacetyl contents of the cultures prepared with the larger amounts of citric acid tended to increase, whereas those prepared with the smaller amounts or none tended to increase at first but often decreased later. The maximum increases in diacetyl of the cultures prepared with the highest concentration of citric acid seemed to be delayed, probably because of slow development of the organisms in a medium which was relatively high in acid when inoculated. At the close of the periods studied, the diacetyl contents were in the same orders as the concentrations of citric acid employed, except with 0.30 per cent in Figure 1.

On the first day the carbinol contents were regularly higher with added citric acid, but the increases again showed no relationship to the amounts of citric acid used. The retarding influence of 0.30 per cent acid was evident, especially in Figure 1. With longer holding the acetylmethylcarbinol contents increased significantly with time, reached a maximum and then decreased, as is well illustrated in Figure 2; presumably the decrease occurred when all the citric acid has been utilized and the carbinol was being converted into such substances as 2,3-butylene glycol and possibly acetic acid. It appears that the larger the amount of citric acid present, the higher the maximum production of the carbinol and the longer the period before the decrease began. If the trial shown in Figure 1 had been extended, the further changes probably would have been somewhat similar to those shown in Figure 2.

The differences in the diacetyl and the carbinol contents in the two trials may have been due to differences in the characteristics of the cultures used. The possible irregularity of some of the data in either of the trials may have been caused by non-uniform shaking of the samples.

In previous studies ripened cultures were usually held for extended periods at 70° F. The results given in Figures 1 and 2, and also in the later figures, show that very substantial increases in both the diacetyl and the acetylmethylcarbinol contents can occur at considerably lower temperatures also.

Series 2. Effects of Shaking Cultures Prepared and Held as in Series 1

Brewer *et al.* (1) found very marked increases in the diacetyl contents by growing butter culture under aeration and pressure, and Virtanen (9) recommended the ripening of butter cultures in shallow containers to allow greater access of air. In analyses (not reported) incidental to the present work it was frequently observed that merely shaking an active culture resulted in an appreciable increase in diacetyl within a relatively short time.

To study the effect of shaking on cultures, trials similar to those in Series 1 were made, but with the difference that in place of only one flask for each concentration of citric acid there were four bottles which were undisturbed until analyzed, as well as one flask from which successive portions were removed for analysis and the remainder then vigorously shaken. The first analyses were made when the cultures had been in the cooler about 6 hours. The results are given in Figures 3 and 4.

The most important points are that the diacetyl contents of the shaken samples increased progressively even without added citric acid, whereas those of the corresponding unshaken samples decreased and then remained at a low level, and that there were relatively small differences in the acetylmethylcarbinol contents of the corresponding shaken and unshaken samples although in most cases the latter were the lower. The initial contents of diacetyl and of the carbinol and the changes in the carbinol contents are in general accord with those of Series 1.

The comparatively high diacetyl contents shown in Figure 4 were obtained with butter culture 146, which has repeatedly produced unusually high diacetyl contents under certain conditions; in practice this culture is considered to be coarse, rather than delicate, in flavor.

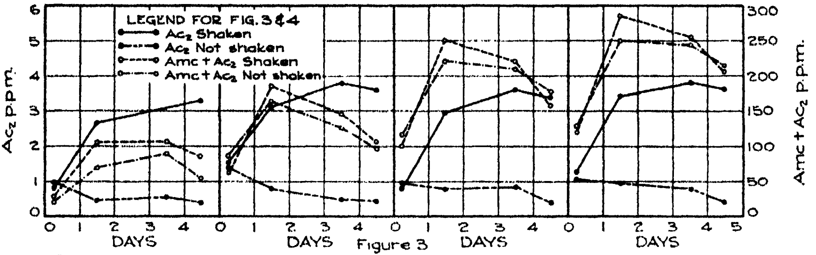

Figure 3

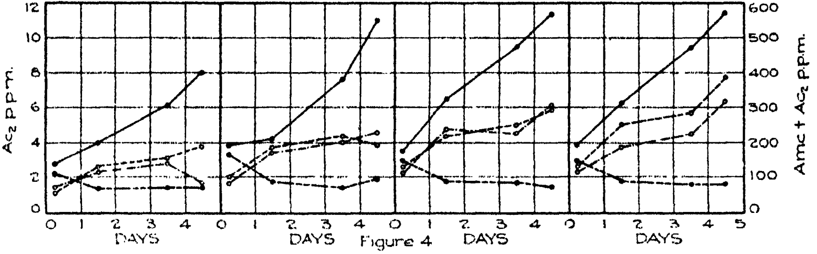

Figure 4

SERIES 2. EFFECTS OF SHAKING CULTURES PREPARED AND HELD AS IN SERIES 1.—The four successive parts of each figure show the values for the diacetyl (Ac$_2$) and the acetylmethylcarbinol plus diacetyl (Amc. + Ac$_2$) when 0.00, 0.10, 0.20, and 0.25 per cent citric acid were used in the preparation of the butter culture.

Series 3. Effect of Periodic Shaking, with or without Simultaneous Addition of Citric Acid, on Ripened Cultures Held Cold

For each trial two large flasks of milk were inoculated, one with no citric acid added to the milk and the other with 0.15 per cent added. Each flask of milk was divided among as many bottles as were required for the analyses to be made. The bottles were incubated at 70° F. and then placed in the cooler. Some of the bottles were given no further treatment, others were shaken, and still others were shaken with addition of 0.08 per cent citric acid; an attempt was made to keep the shaking uniform. The time and nature of these subsequent treatments and the results obtained are indicated in Figures 5, 6, 7, and 8. The analyses on the first day were made from 6 to 10 hours after placing the samples in the cooler. In these figures the analytical results for each sample are shown in a circle. The circles are indicated in perspective as being at different levels to suggest the relative values of the diacetyl contents most commonly obtained under the various conditions indicated. Naturally, a few of the diacetyl values in the individual trials show departures from the general tendencies, and also the changes in the acetylmethylcarbinol contents frequently do not parallel the changes in the diacetyl contents.

The diacetyl contents of the cultures held without treatment often increased somewhat from the first to the second day and in some cases to the third day. This may have been influenced by a small amount of oxygen

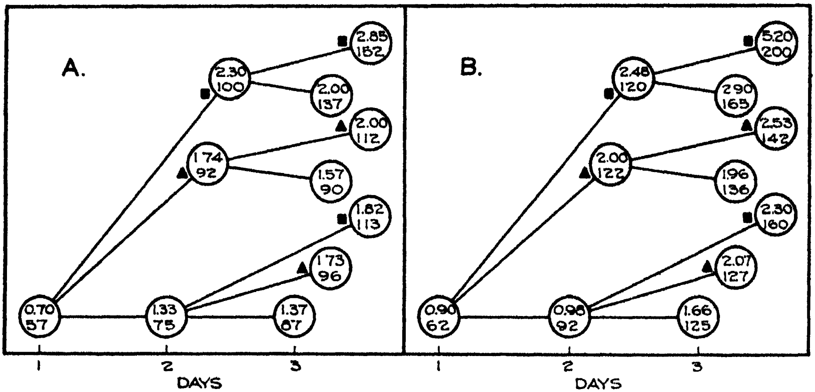

Figure 5

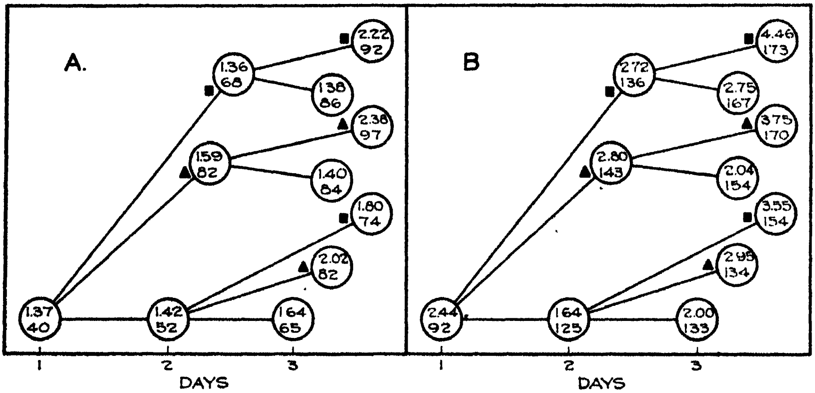

Figure 6

▲ Shaken one day before analysis ■ 0.08% Citric acid added with shaking one day before analysis

SERIES 3. EFFECTS OF PERIODIC SHAKING, WITH OR WITHOUT SIMULTANEOUS ADDITION OF CITRIC ACID, ON RIPENED CULTURES HELD COLD.—A. No citric acid added to original milk. B. 0.15 per cent citric acid added. Top figure in each circle = p.p.m. diacetyl and bottom figure = p.p.m. acetylmethylcarbinol plus diacetyl.

still present and possibly by other factors. The cultures shaken on the first day showed markedly increased diacetyl contents on the second day and part of such increases were usually retained on the third day; when shaken on both the first and second days there were still higher diacetyl contents on the third day. The cultures shaken with the addition of citric acid on the first day gave greatly increased diacetyl contents on the second day which were commonly retained on the third day; by using this treatment on both the first and second days the highest diacetyl contents were usually attained on the third day. In most cases the cultures prepared with citric acid added to the milk originally had higher diacetyl contents than the

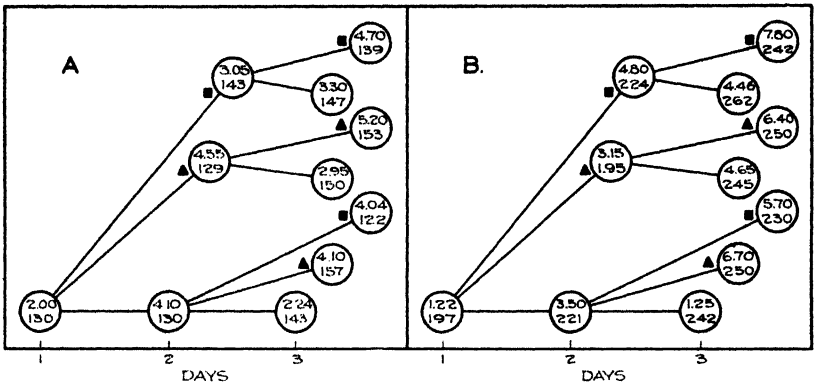

Figure 7

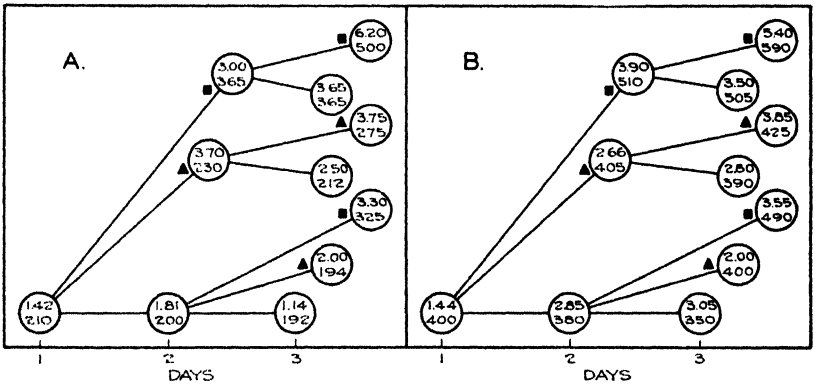

Figure 8

▲ Shaken one day before analysis ■ 0.08% Citric acid added with shaking one day before analysis

Series 3, *continued.* Effects of Periodic Shaking, with or without Simultaneous Addition of Citric Acid, on Ripened Cultures Held Cold.—A. No citric acid added to original milk. B. 0.15 per cent citric acid added. Top figure in each circle = p.p.m. diacetyl and bottom figure = p.p.m. acetylmethylcarbinol plus diacetyl.

corresponding cultures without such addition. Some of the samples held without treatment subsequent to ripening were among the exceptions; in these cases the available oxygen rather than the citric acid may have been the limiting factor.

The acetylmethylcarbinol contents increased during holding without any treatment, but shaking or shaking with addition of citric acid usually gave additional increases. The increases in the carbinol contents that resulted from shaking may have been due to prevention of reduction to 2,3-butylene glycol (3), although there are other possibilities.

For the data shown in Figure 8 the ripened cultures were held at a slightly higher temperature (42° to 50° F.) than usual. The high acetylmethylcarbinol contents on the first day suggest that there already had been extensive utilization of the citric acid. The subsequent addition of citric acid caused very substantial increases in both diacetyl and the carbinol but especially in the carbinol; presumably, this was due to the higher temperature and the greater activity of the organisms used.

Relatively high diacetyl contents are shown in Figure 7. These were again obtained with butter culture 146. However, the acetylmethylcarbinol contents were not greatly influenced by the treatments subsequent to ripening.

Series 4. Effects of Adding Citric or Sulfuric Acid to Ripened Butter Cultures Held at 70° F. or Packed in Ice

For each trial butter culture was prepared with and without addition of citric acid to the original milk. Each lot of ripened culture was then divided among a number of bottles and various amounts of citric or sulfuric acid added to the different bottles with uniform shaking. The pH values were determined with a quinhydrone electrode. Some of the bottles in each trial were returned to 70° F. and some were packed in ice. The data are given in Table 1.

Two trials, 1 and 2, show the effects of 0.15 per cent citric acid added to the milk used for the cultures. The various treatments of the ripened cultures commonly gave higher diacetyl and acetylmethylcarbinol contents when an original addition of citric acid had been made. These results substantiate those already presented.

With the addition of citric acid to the ripened cultures held at 70° F., both the diacetyl and the carbinol contents were progressively higher the greater the percentage of citric acid added; when the cultures were held in ice the diacetyl contents usually were even higher than those of the corresponding samples held at 70° F., but there commonly was relatively little production of the carbinol.

The addition of sulfuric acid to the ripened culture held at 70° F. resulted in increases in the diacetyl and in the carbinol, but these increases were considerably less than those in cultures having similar initial pH values as the result of adding citric acid. However, when the samples with sulfuric acid were held in ice the diacetyl contents were often higher than the corresponding values obtained with citric acid.

The results with sulfuric acid emphasize the importance of pH from the standpoint of production of diacetyl and also of the carbinol, since sulfuric acid cannot be attacked by the organisms. Michaelian, Hoecker and Hammer (6) found that with milk cultures of citric acid fermenting streptococci acidified with certain acids there were definite optimum pH ranges (3.9 to 3.7 with citric, 4.4 to 4.1 with lactic and 3.6 to 3.2 with sulfuric acid) for the maximum production of the carbinol plus diacetyl

TABLE 1

Effect of adding citric or sulfuric acid to ripened butter cultures held at 70° F. or packed in ice on diacetyl (Ac_2) and acetylmethylcarbinol plus diacetyl ($Amc + Ac_2$) contents

Trial no.		1						2				3			4			5		
		a			b			a		b										
Butter culture used, no.		15/1						232				232			232			15/1		
Citric acid added before ripening culture		none			0.15 per cent			none		0.15 per cent		none			0.15 per cent			0.15 per cent		
Treatment of ripened culture		pH*	p.p.m.		pH	p.p.m.		p.p.m.		p.p.m.		pH	p.p.m.		pH	p.p.m.		pH	p.p.m.	
Holding conditions	Per cent acid added		Ac_2	$Amc + Ac_2$		Ac_2	$Amc + Ac_2$	Ac_2	$Amc + Ac_2$	Ac_2	$Amc + Ac_2$		Ac_2	$Amc + Ac_2$		Ac_2	$Amc + Ac_2$		Ac_2	$Amc + Ac_2$
Analysed at once	none	4.55	0.36	87.	4.55	0.63	210.	0.65	87.	0.89	175.	...	...	...	...	...	...	...	...	...
Held at 70° F. for 9–12 hrs.	none	4.55	0.90	70.	4.55	1.30	208.	1.12	112.	1.90	250.	4.50	1.15	99.	4.45	1.10	240.	4.63	0.95	140.
	citric 0.08	4.38	2.00	186.	4.36	1.95	345.	2.00	232.	3.10	410.	...	...	...	...	...	...	...	...	...
	.15	...	...	...	...	...	...	...	...	...	...	4.31	2.00	266.	4.12	5.35	458.	4.30	2.80	332.
	.25	4.09	3.32	257.	4.08	3.40	515.	3.53	208.	4.30	492.	4.07	3.10	250.	...	...	...	...	...	...
	.35	...	...	...	...	...	...	...	...	...	...	3.98	4.70	210.	3.89	9.60	580.	4.00	5.00	385.
	.45	...	...	...	...	...	...	...	...	...	...	3.89	6.50	200.	...	...	...	...	...	...
	sulfuric 0.17	...	...	...	...	...	...	...	...	...	...	...	...	...	4.08	1.90	248.	4.27	2.10	182.
	.23	4.11	1.45	100.	4.11	1.87	313.	2.50	154.	3.85	357.	...	...	...	...	...	...	...	...	...
	.30	...	...	...	...	...	...	...	...	...	...	...	...	...	3.85	2.00	258.	4.04	3.00	182.
Held in ice for 12–15 hrs.	none	4.55	2.65	87.	4.55	3.00	238.	2.65	123.	2.06	200.	4.50	3.05	120.	4.45	2.60	255.	4.63	3.25	177.
	citric 0.08	4.38	3.32	148.	4.36	4.40	285.	3.70	127.	5.00	240.	...	...	...	...	...	...	...	...	...
	.15	...	...	...	...	...	...	...	...	...	...	4.31	4.15	137.	4.12	7.00	357.	4.30	4.15	230.
	.25	4.09	3.95	125.	4.08	5.20	263.	3.95	114.	6.40	188.	4.07	5.17	133.	...	...	...	...	...	...
	.35	...	...	...	...	...	...	...	...	...	...	3.98	5.35	120.	3.89	8.00	345.	4.00	5.65	220.
	.45	...	...	...	...	...	...	...	...	...	...	3.87	5.55	113.	...	...	...	...	...	...
	sulfuric 0.17	...	...	...	...	...	...	...	...	...	...	...	...	...	4.08	4.70	266.	4.27	4.07	200.
	.23	4.11	4.15	103.	4.15	5.00	264.	5.00	114.	6.00	210.	...	...	...	...	...	...	...	...	...
	.30	...	...	...	...	...	...	...	...	...	...	...	...	...	3.85	5.35	250.	4.04	4.85	200.

* pH taken after adding acid to ripened cultures.

(obviously pH optima for acetylmethylcarbinol production since this compound comprises all but a small fraction of the mixture). Apparently there is also a definite optimum pH for production of diacetyl with sulfuric or citric acid which seems to be lower than the pH developed in a normal butter culture. Addition of citric acid to a ripened culture also lowers the pH, but the data indicate its chief rôle is to supply the parent substance for diacetyl and the carbinol.

DISCUSSION

Various factors tended to give relatively high diacetyl contents in butter cultures. A consideration of these suggests that while a supply of citric acid is important, an oxygen supply also is of significance. Evidently, there is some oxidation reaction involved in the formation of diacetyl, and oxygen also may prevent the reduction of this compound. Since shaking was effective in this connection, the normal agitation of a culture in a butter plant probably has a value other than simply breaking up the curd. The effectiveness of extreme aeration is shown by the work of Brewer *et al.* (1). The acetylmethylcarbinol contents are influenced by the same general factors affecting the diacetyl contents and in this connection oxygen presumably tends to prevent reduction to 2,3-butylene glycol.

Analytical results on successive days for ripened culture held cold show that changes in the diacetyl and the carbinol contents can occur at temperatures considerably below those used for ripening cultures. The addition of citric acid to the cultures held cold tended to give increases in both these products which emphasizes the importance of this acid in the fermentation. These results are in agreement with practices in those plants which regularly hold ripened butter culture cold at least 24 hours before using it in cream.

The pH is a factor influencing the diacetyl and acetylmethylcarbinol contents of cultures. Apparently, the most favorable pH values are lower than the pH range of normal butter cultures, but possibly they vary with temperature.

A high diacetyl content would be assumed to accompany a high acetylmethylcarbinol content but various factors, such as the oxygen supply, temperature, absence of citric acid, etc., may prevent establishment of any close relationship.

SUMMARY

In studies on the changes in the diacetyl and the acetylmethylcarbinol contents of butter cultures under a variety of conditions, the factors found to favor a high diacetyl content were:

1. Addition of citric acid to the milk used. About 0.15 per cent citric acid appeared to be a practical amount. Larger percentages, such as 0.30

per cent, did not give correspondingly higher diacetyl contents unless the ripened cultures were held cold for an extended period with sufficient aeration.

2. Aeration of the cultures. Shaking the ripened cultures produced significant increases in diacetyl and lack of aeration caused decreases. Under practical conditions, normal agitation undoubtedly gives the same effect.

3. Holding the ripened cultures. There were commonly increases in diacetyl during the holding of cultures at various temperatures, but holding in the cooler is apparently the most practical.

4. Addition of citric acid to the ripened cultures. As little as 0.08 per cent was effective, the agitation incident to the distribution presumably also being a factor.

5. Production of a low pH in the ripened cultures. Lowering the pH of ripened cultures, even with such an acid as sulfuric, tended to increase the diacetyl content.

In general, a high acetylmethylcarbinol content was favored by similar conditions. The attainment of a relatively high content at low temperatures is slower with the carbinol than with diacetyl.

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CHANGES IN THE DIACETYL AND THE ACETYLMETHYLCARBINOL CONTENTS DURING THE MANUFACTURE OF BUTTER[1]

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Results of various investigations indicate that diacetyl is an important flavor constituent of butter. It is contributed by the butter culture used and by the cream. Apparently, in the fermentation characteristic of butter cultures the diacetyl is formed through an oxidation of acetylmethylcarbinol which is produced from citric acid, both the production and oxidation being brought about primarily by the citric acid fermenting streptococci (*Streptococcus citrovorus* and *Streptococcus paracitrovorus*) normally present. Sour cream contains diacetyl and acetylmethylcarbinol as a result of bacterial action, but traces of these products also appear to be present in sweet cream which presumably has not undergone any considerable change due to organisms.

Since butter culture and cream contain much less diacetyl than acetylmethylcarbinol, and also because often citric acid is still present, there is an opportunity for changes in the diacetyl and the carbinol contents during the manufacture of butter. Data on this point are presented herein.

METHODS

Manufacturing Procedure

The butter studied was made during the spring and early summer of 1938. The cream was delivered to the butter laboratory of the Iowa State College from farms in the vicinity, and was made into butter in the usual operation of the laboratory[3] (6).

Both the sweet and sour cream contained approximately 30 per cent fat. The cream was pasteurized at 150° F. for 30 minutes, the sour cream being neutralized to about 0.25 per cent acid prior to pasteurization; following cooling, both the sweet and sour cream were neutralized to approximately 0.10 per cent acid. The churnings in which aerated or modified aerated butter culture was used and some of those in which regular butter culture was employed with sweet cream were made with 150 lb. portions of cream, while the remainder were of commercial size.

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[3] The churnings were made under the supervision of Dr. N. E. Fabricius who is in charge of the butter laboratory.

Analytical Procedures

Several investigators have reported on certain aspects of the changes in diacetyl and acetylmethylcarbinol contents during the manufacture of butter. Barnicoat (1) used his colorimetric method (2) which is based on a comparison of the yellow chloroform solutions of nickel dimethylglyoximate, Mohr and Wellm (9) employed their modification (10) of this procedure, Brioux and Jouis (4) made use of the colorimetric diaminobenzidine method (11), while Fabricius and Hammer (6), Davies (5), Krenn (8), and Virtanen (14) employed some form of the gravimetric procedure. The results reported by these investigators do not permit of close comparisons because of differences in the methods of buttermaking in the various countries and perhaps also because of the different analytical methods used.

In the present work the diacetyl was conveniently determined by the colorimetric procedure recently developed by Prill and Hammer (12) which was designed to avoid the difficulties encountered with some of the other methods, particularly when relatively small amounts of diacetyl are involved. The principle of the method is the conversion of the diacetyl into dimethylglyoxime and then into the intensely colored ammono-ferrous dimethylglyoximate.

The acetylmethylcarbinol plus diacetyl was also determined colorimetrically. Small aliquots of the distillate from the cream or culture distilled with added ferric chloride were used under special conditions (13). Samples of butter (20 g. each) with ferric chloride added were distilled in a special apparatus (12).

The results are recorded as milligrams of diacetyl or of acetylmethylcarbinol plus diacetyl per kilogram of the sample, which is synonymous with expressing them as parts per million.

RESULTS

In some of the first trials it was noted that the neutralized and pasteurized cream frequently had a lower diacetyl content than the raw cream and, accordingly, a more detailed study of this point was made with several lots of sour cream. The results, which are shown in Table 1, indicate some rather unexpected changes. Shortly after the preliminary neutralization of the cream to 0.25 per cent acid (the original acidity was often 0.5 to 0.6 per cent), or during the first part of the heating, the diacetyl content fell to a very low value and the carbinol content commonly decreased slightly. This may have been due to a reduction of these compounds to 2,3-butylene glycol (7) in the less acid medium. When the cream had been heated to pasteurizing temperature and in the subsequent tests the diacetyl contents showed very significant increases which were probably actually even greater than shown by the analyses because of loss through

TABLE 1

Changes in diacetyl and acetylmethylcarbinol contents during neutralization and pasteurization of sour cream

Cream	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Milligrams per kilogram									
	Ac_2*	Amc† $+Ac_2$	Ac_2	Amc $+Ac_2$	Ac_2	Amc $+Ac_2$	Ac_2	Amc $+Ac_2$	Ac_2	Amc† $+Ac_2$
Raw	1.11	39.0	0.61	37.0	0.71	22.0	0.94	26.0	1.10	27.0
Neut. to 0.25% acidity	.24	37.0	.15	24.0	.20	26.0	.30	26.0	.10	29.0
Heated to 100° F.							.16	23.0	.12	22.0
Heated to 150° F.							.55	33.0	.39	27.0
After holding 30 min.							.52	32.0	.43	29.0
Cooled					.48	28.0	.75	33.0	.48	30.0
After final neutralization to 0.10% acidity			.55	36.0	.56	28.0	.74	32.0	.43	29.0
After holding about 1 hr.	.51	40.0	.35	32.0			.86	32.0	.57	28.0

* Ac_2 = diacetyl.
† Amc = acetylmethylcarbinol.

volatilization. The carbinol content showed an increase when the cream had reached the pasteurizing temperature and then remained fairly constant in the subsequent tests. The increase in diacetyl may possibly have been caused by non-biological oxidation but the increase in the carbinol through non-biological reactions seems improbable. The net result of the processing of the cream was a decrease in the diacetyl content and relatively little change in the acetylmethylcarbinol content.

Barnicoat (1) reported that during neutralization and pasteurization of cream (presumably the flash method) the carbinol is partly destroyed while the diacetyl is practically unchanged. Apparently, generalizations on these points which are applicable to all methods of processing cream cannot be made at present.

For the churnings used in studying the changes in the diacetyl and acetylmethylcarbinol contents, the butter culture was made with addition of 0.15 per cent citric acid (monohydrate) to the skim or whole milk employed, and from 5 to 10 per cent was added to the pasteurized and cooled cream. Except in one case, which is indicated, the mixture was held cold over night (36° to 44° F. so there would be no significant increase in acid) and churned the next morning. Samples of the processed cream, the butter culture, the mixture of cream and culture immediately after its preparation, the mixture just before churning, the mixture partly churned, the buttermilk, and the finished salted butter were analysed within 1 or 2 hours; they were kept cold during this interval. From the percentages of culture used and on the basis that 30 per cent cream plus 5 to 10 per cent culture yields approximately 1 part of butter (80 per cent fat) and 2 parts of buttermilk, the milligrams of diacetyl and of the carbinol plus

TABLE 2

Changes in diacetyl and acetylmethylcarbinol contents during manufacture of butter from sweet cream with regular culture added

Trial no.	Per cent culture added to cream	Compounds determined	Diacetyl (Ac_2) and acetylmethylcarbinol plus diacetyl ($Amc + Ac_2$) in mg. per kg.							Estimated per cent of the total Ac_2 or $Amc + Ac_2$ retained in butter
			Past. neut. cream	Butter culture	Cream plus culture			Buttermilk	Butter	
					at once	just before churning	partly churned			
1	10	Ac_2	.03 *(.03)*[*]	1.53 *(.15)*	.32			1.60 *(1.07)*	.33 *(.11)*	
		$Amc + Ac_2$	5.1 *(4.6)*	315. *(31.5)*	34.			58. *(39.)*	8.0 *(2.7)*	
2	10	Ac_2	.10 *(.09)*	.69 *(.07)*	.39	.80	.82	1.33 *(.89)*	.28 *(.09)*	11.0
		$Amc + Ac_2$	2.8 *(2.5)*	278. *(28.)*	27.	37.		46. *(31.)*	5.5 *(1.8)*	4.9
3	7	Ac_2	.08 *(.07)*	.90 *(.06)*	.17	.26	.71	.81 *(.54)*	.14 *(.05)*	7.0
		$Amc + Ac_2$	3.3 *(3.1)*	270. *(19.)*	20.	21.	18.	27. *(18.)*		
4	10	Ac_2	.08 *(.07)*	.90 *(.09)*	.24	.50	.62	.86 *(.57)*	.18 *(.06)*	9.7
		$Amc + Ac_2$	3.3 *(3.0)*	270. *(27.)*	30.	30.		51. *(34.)*		
5	7	Ac_2	.18 *(.17)*	.83 *(.06)*	.32	.56	.60	.96 *(.64)*	.18 *(.06)*	10.0
		$Amc + Ac_2$	7.7 *(7.1)*	300. *(21.)*	24.	26.		38. *(24.)*	4.0 *(1.3)*	5.1
6	7	Ac_2	.25 *(.23)*	.52 *(.04)*	.42	1.18		1.66 *(1.10)*	.30 *(.10)*	
		$Amc + Ac_2$	12.5 *(11.6)*	232. *(16.)*	30.	59.		70. *(47.)*	5.5 *(1.8)*	3.6
7	10	Ac_2	.16 *(.14)*	2.56 *(.26)*	.41	.54	.61	.61 *(.40)*	.18 *(.06)*	9.8
		$Amc + Ac_2$	7.7 *(6.9)*	147. *(15.)*	24.	25.		30. *(20.)*	3.6 *(1.2)*	4.8
8	10	Ac_2	.05 *(.04)*	3.60 *(.36)*	.47	.83	.97	1.35 *(.90)*	.21 *(.07)*	7.2
		$Amc + Ac_2$	2.5 *(2.2)*	260. *(26.)*	29.	36.		53. *(35.)*		
9	10	Ac_2	.09 *(.08)*	2.16 *(.22)*	.49	1.05	1.17	1.47 *(.97)*	.29 *(.10)*	8.5
		$Amc + Ac_2$	3.7 *(3.3)*	306. *(31.)*	35.	49.		68. *(45.)*	5.8 *(1.9)*	3.9
10	10	Ac_2	.10 *(.09)*	.77 *(.08)*	.40	1.05	1.10	1.66 *(1.10)*	.33 *(.11)*	10.0
		$Amc + Ac_2$	4.2 *(3.8)*	360. *(36.)*	39.	56.		85. *(56.)*	7.8 *(2.6)*	4.7
11	10	Ac_2	.33 *(.30)*	.70 *(.07)*	.87	1.37	1.55	1.97 *(1.31)*	.38 *(.13)*	8.1
		$Amc + Ac_2$	16. *(14.5)*	250. *(25.)*	47.	60.	61.	74. *(49.)*	7.3 *(2.4)*	4.0
12	10	Ac_2	.09 *(.08)*	.56 *(.06)*	.32	1.30	1.64	1.48 *(1.00)*	.36 *(.12)*	7.3
		$Amc + Ac_2$	3.9 *(3.5)*	257. *(26.)*	26.	53.		74. *(49.)*	7.9 *(2.7)*	5.1

[*] Numbers in parentheses and italics denote the estimated mg. of Ac_2 or of $Amc + Ac_2$ contributed to, or derived from, 1 kg. of the churning mixture by the materials indicated.

diacetyl contributed to, or derived from, 1 kg. of the churning mixture were estimated. The percentages of the diacetyl and of the carbinol plus diacetyl in the churning mixtures that were retained in the butter were also estimated.

Data obtained when regular butter culture was used with sweet cream are given in Table 2. The mixture of cream and culture at once usually contained approximately the amount of acetylmethylcarbinol contributed by the cream and the culture, whereas the diacetyl content was regularly higher than the calculated amount. The incorporation of air during the mixing presumably made it possible for the organisms to effect considerable oxidation. The next morning the diacetyl had increased further, often more than doubling over night, and the carbinol also showed some increases but the percentage increases were not as great as in the case of the diacetyl. During churning there were further increases in diacetyl; the carbinol, however, would not be expected to change greatly in the short period involved.

Fabricius and Hammer (6) found that when mixtures of cream and culture were held for a time before churning, the acetylmethylcarbinol plus diacetyl contents increased and the resulting butter scored higher than butter made by churning soon after the addition of the culture. Other investigators (8, 9, 14) also noted significant increases in the diacetyl and the carbinol contents during holding and churning of cream. Recent work (13) shows that similar increases occur in butter culture under analogous treatments.

The diacetyl and acetylmethylcarbinol contents of the butter and buttermilk are considered later with the values from the other series of churnings.

Table 3 presents results obtained when regular culture was used with sour cream. More diacetyl was contributed to the mixtures of cream and culture by the neutralized and pasteurized cream than by the regular culture, and acetylmethylcarbinol was contributed in comparable amounts from the two sources. However, the culture supplied active organisms which made possible a further accumulation of these two products. Except for a few irregularities, the changes occurring in the diacetyl and acetylmethylcarbinol contents were similar to those obtained with mixtures of sweet cream and regular culture.

Results obtained when aerated (3) or modified aerated cultures were used with sweet cream are given in Table 4. The aerated cultures were grown with aeration under pressure for 12 to 13 hours and had extremely high diacetyl contents (up to 37.4 mg. per kg.); the acetylmethylcarbinol contents were also much higher than those of regular cultures. The modified aerated cultures were aerated under pressure for 3 hours after the usual ripening period; they contained relatively large amounts of diacetyl and the carbinol contents were somewhat higher than those of regular

TABLE 3

Changes in diacetyl and acetylmethylcarbinol contents during manufacture of butter from sour cream with regular culture added

Trial no.	Per cent culture added to cream	Compounds determined	Diacetyl (Ac_2) and acetylmethylcarbinol plus diacetyl ($Amc + Ac_2$) in mg. per kg.							Estimated per cent of the total Ac_2 or $Amc + Ac_2$ retained in butter
			Past. neut. cream	Butter culture	Cream plus culture			Buttermilk	Butter	
					at once	just before churning	partly churned			
1	5	Ac_2	.54 *(.52)**	.94 *(.05)*	.54	.42	1.00	1.25 *(.84)*	.20 *(.07)*	6.7
		$Amc + Ac_2$	7.7 *(7.3)*	250. *(13.)*	27.	39.		55. *(37.)*	6.1 *(2.1)*	5.3
2†	10	Ac_2	.15 *(.14)*	.65 *(.07)*	.70	.79		1.18 *(.79)*	.37 *(.12)*	
		$Amc + Ac_2$	9.6 *(8.7)*	305. *(31.)*	47.	55.		60. *(40.)*	5.5 *(1.8)*	3.3
3	5	Ac_2	.43 *(.41)*	.33 *(.02)*	.47	1.13	1.20	1.20 *(.80)*	.32 *(.11)*	8.9
		$Amc + Ac_2$	20.7 *(19.6)*	230. *(12.)*	38.	42.	40.	52. *(35.5)*	5.3 *(1.8)*	4.5
4	5	Ac_2	.36 *(.34)*		.38	.36	.43	.55 *(.37)*	.16 *(.05)*	12.3
		$Amc + Ac_2$	14.5 *(13.8)*		20.	17.	15.	21. *(14.)*	2.8 *(.9)*	6.2
5	5	Ac_2	.40 *(.38)*	.38 *(.02)*	.52	.51	1.05	1.22 *(.82)*	.27 *(.09)*	8.5
		$Amc + Ac_2$	19. *(18.)*	266. *(13.)*	30.	46.		74. *(50.)*	5.0 *(1.7)*	3.7
6	5	Ac_2	.47 *(.45)*	.59 *(.03)*	.46	1.00		1.07 *(.72)*	.36 *(.12)*	
		$Amc + Ac_2$	28. *(27.)*	270. *(14.)*	39.5	53.		74. *(50.)*	6.5 *(3.2)*	6.0
7	7	Ac_2	.51 *(.47)*	.34 *(.03)*	.46	1.10	1.08	1.10 *(.73)*	.40 *(.13)*	12.0
		$Amc + Ac_2$	40.5 *(37.)*	262. *(18.)*	51.	60.		79. *(53.)*	7.1 *(2.4)*	4.0
8	8	Ac_2	.35 *(.32)*	.33 *(.03)*	.66		1.00	1.10 *(.73)*	.25 *(.08)*	8.3
		$Amc + Ac_2$	32. *(29.)*	130. *(10.)*	40.		41.	55. *(37.)*	4.4 *(1.5)*	3.6

* See footnote of Table 2.

† In this trial cream was prepared and churned the same day.

cultures. Analyses of the mixtures of cream and culture at once do not indicate an increase in diacetyl during mixing but rather some of them suggest a slight decrease, especially in the case of the aerated cultures. It appears that after having grown in a strongly aerated medium the organisms did not immediately effect further oxidations leading to production of diacetyl on transfer to the less aerated medium. On holding until the next morning and during churning, the organisms usually produced additional diacetyl and acetylmethylcarbinol and although, in most cases, the actual increment in diacetyl was as great (or greater) as with regular culture, the percentage increase was not, because of the relatively large amount of diacetyl originally present. Consequently, in comparisons involving the addition of equal percentages of regular and aerated or modified aerated culture to pasteurized sweet cream, the butter made with the latter types of culture invariably had the higher diacetyl content (0.38 to 1.00 mg. per kg. as compared to 0.14 to 0.38 mg. per kg.), but these higher values were really much lower than they would be if the diacetyl contents of the butter were proportional to those of the cultures used. In case the cream is churned soon after the addition of the culture, however, aerated or modified aerated cultures probably would show a greater practical advantage over regular cultures.

In the distribution of the diacetyl and the acetylmethylcarbinol between the butter and the buttermilk, certain general relationships apply to all the churnings studied. The estimated milligrams of diacetyl in the buttermilk and the butter derived from 1 kg. of the churning mixture agree fairly well, in most cases, with the diacetyl content of the partly churned mixture. Also, the estimated milligrams of the carbinol plus diacetyl in the two products agree fairly well with the content just before churning. Except for a few irregularities, the diacetyl contents of the butter were very nearly 1/5 of the diacetyl contents of the corresponding buttermilk. Since butter contains about 1/5 serum it would appear at first sight that the diacetyl is contained entirely in the serum of the butter. However, the carbinol plus diacetyl content of the butter was, in most cases, only about 1/10 that of the buttermilk, which is much less than would be expected because acetylmethylcarbinol is presumably even more preferentially dissolved in the water phase than is the diacetyl. The average of the estimated percentages of the diacetyl in the churning mixtures that were retained in the butter was 8.8 per cent, and the corresponding value for the carbinol plus diacetyl was 4.5 per cent.

Barnicoat (1) reported that 0 to 33 per cent (average 15 per cent) of the diacetyl and 7 to 22 per cent (average 14 per cent) of the carbinol in cream were retained in the butter, and on the basis of the averages of these divergent values postulated that diacetyl and the carbinol were contained in the butter serum. Actually, a number of his analyses of individual churnings

TABLE 4

Changes in diacetyl and acetylmethylcarbinol contents during manufacture of butter from sweet cream with aerated or modified aerated culture added

Trial no.	Per cent and type culture added to cream	Compounds determined	Diacetyl (Ac_2) and acetylmethylcarbinol plus diacetyl ($Amc + Ac_2$) in mg. per kg.							Estimated per cent of the total Ac_2 or $Amc + Ac_2$ retained in butter
			Past. neut. cream	Butter culture	Cream plus culture			Buttermilk	Butter	
					at once	just before churning	partly churned			
1	10 a†	Ac_2	.10 (*.09*)*	16.8 (*1.68*)	1.55	1.39	2.22	2.44 (*1.65*)	.40 (*.13*)	6.0
		$Amc + Ac_2$	2.8 (*2.5*)	410. (*41.*)	36.	34.		47. (*31.*)	4.8 (*1.6*)	4.7
2	10 a	Ac_2	.08 (*.07*)	25.0 (*2.50*)	2.30	2.40	2.90	3.60 (*2.40*)	.60 (*.20*)	6.9
		$Amc + Ac_2$	3.3 (*3.0*)	570. (*57.*)	54.	68.	68.	69. (*46.*)		
3	10 a	Ac_2	.16 (*.14*)	26.8 (*2.68*)	2.60	3.25	3.70	3.90 (*2.60*)	1.00 (*.33*)	9.0
		$Amc + Ac_2$	7.7 (*6.9*)	750. (*75.*)		90.		118. (*79.*)	13.4 (*4.5*)	5.0
4	10 a	Ac_2	.05 (*.04*)	37.4 (*3.74*)	3.85	3.85	3.35	5.30 (*3.52*)	.93 (*.31*)	9.3
		$Amc + Ac_2$	2.5 (*2.2*)	920. (*92.*)	85.	97.		120. (*80.*)		
5	10 ma‡	Ac_2	.09 (*.08*)	12.7 (*1.27*)	1.43	1.58	1.43	2.31 (*1.54*)	.44 (*.15*)	10.2
		$Amc + Ac_2$	3.7 (*3.3*)	410. (*41.*)	43.	46.		72. (*48.*)	6.5 (*2.2*)	4.8
6	10 ma	Ac_2	.10 (*.09*)	10.0 (*1.00*)	.91	1.14	1.40	2.00 (*1.33*)	.38 (*.13*)	9.0
		$Amc + Ac_2$	4.2 (*3.8*)	308. (*31.*)	35.	40.		59. (*39.*)	5.6 (*1.8*)	4.7
7	10 ma	Ac_2	.33 (*.30*)	15.5 (*1.55*)	1.96	2.60	2.72	3.90 (*2.60*)	.68 (*.23*)	8.5
		$Amc + Ac_2$	16.0 (*14.5*)	417. (*42.*)	53.	75.	74	98. (*65.*)	9.3 (*3.1*)	4.2
8	10 ma	Ac_2	.09 (*.08*)	17.5 (*1.75*)	1.47	2.22	2.70	2.72 (*1.81*)	.56 (*.19*)	6.9
		$Amc + Ac_2$	3.9 (*3.5*)	323. (*32.*)	37.	62.	69.	82. (*55.*)	6.8 (*2.3*)	3.3

* See footnote of Table 2.

† a = aerated butter culture.

‡ ma = modified aerated butter culture.

showed a ratio of approximately 2 to 1 for the percentages of diacetyl and of the carbinol retained in the butter. The results obtained by Mohr and Wellm (9) indicate a lower percentage retention of diacetyl and a still lower percentage retention of the carbinol. They attributed this to the removal of some of these products by the wash water, presumably, relatively more of the carbinol than diacetyl being washed out. Krenn (8) made similar claims in regard to the washing. In the present work the analyses of a few samples of wash water showed only fractions of a milligram of the carbinol plus diacetyl per kilogram. While the total amount present in the wash water may be significant in comparison with the amount present in the butter, it is difficult to differentiate between material which comes from the film of buttermilk remaining between the butter granules and that which might possibly be removed from the granules themselves. If the diacetyl and the carbinol are both held entirely in the serum droplets in the same manner, they should be retained in the butter in the same proportion as they exist in the churning mixture at the time the butter is formed, and any possible washing out of these products should also be in this proportion. Actually, the distribution of the diacetyl and the carbinol in butter is probably more complex than has been assumed. The serum in the form of minute droplets may possibly have solvent properties different from those of larger volumes of this fluid. The possibility of the adsorption of the diacetyl at the enormous water-fat interface should also be considered, as well as the possibility of the solution of a portion of the diacetyl in the fat.

With the churnings made by adding any of the types of culture to sweet cream it was not possible to note a close relationship between the diacetyl contents of the culture and of the finished butter. Undoubtedly other factors, such as acetylmethylcarbinol content, acidity, oxygen tension, and numbers and activity of the organisms present are also involved. It would be even more difficult to detect a relationship when sour cream is used because of the greater variation in the quality of such cream and the comparatively large amount of diacetyl it contributes to the mixture of cream and culture.

SUMMARY

Marked changes in diacetyl content, both decreases and increases, were noted during the neutralization and pasteurization of sour cream. The changes in the acetylmethylcarbinol content were relatively small.

Progressive increases in diacetyl were found after mixing regular butter culture with pasteurized cream (either sweet or sour), after holding the mixture at a low temperature over night, and after the mixture had been partly churned. The acetylmethylcarbinol content increased somewhat during the holding period. The results when aerated or modified aerated butter culture was added to sweet cream were similar to those when regular culture was employed, except that no increases in diacetyl were noted immediately after mixing, and the subsequent increases, while quite significant in actual amounts, were small on a percentage basis because of the relatively large amounts of diacetyl already supplied by these cultures.

It was not possible to note a close relationship between the diacetyl contents of the butter cultures added to the cream and those of the resulting butter. Aerated or modified aerated cultures, as compared with equal percentages of regular cultures, yielded butter having higher diacetyl contents, but these values were really much lower than they would have been if a proportionality existed between the diacetyl contents of the cultures and the butter.

On the average, 8.8 per cent of the diacetyl and 4.5 per cent of the acetylmethylcarbinol plus diacetyl in the churning mixture were retained in the butter.

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EFFECTS OF THE COLD STORAGE TEMPERATURE, HEAT TREATMENT, AND HOMOGENIZATION PRESSURE ON THE PROPERTIES OF FROZEN CONDENSED MILK

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The effects of freezing on whole milk and condensed milk have been studied by numerous investigators. Webb and Hall (1) studied primarily the physical effects of freezing and storage, and suggested the use of frozen condensed milk as a source of fresh market milk where the latter is expensive or not available. More recent studies of the freezing of milk and milk products have been made by Baldwin and Doan (2), Doan and Baldwin (3), Doan and Featherman (4), and Heiss (5).

The work of Webb and Hall has been extended in order to ascertain more in detail the desirable features of the process for preparing and preserving frozen condensed milk. Concentration milks of not more than 8.0 per cent fat content were prepared, and the factors investigated were those which must be considered in the commercial development of frozen condensed milk.

EFFECT OF STORAGE TEMPERATURE

The milk was produced by the Bureau of Dairy Industry herd at Beltsville, Maryland, and handled in the dairy plant there. There was opportunity for it to dissolve copper from equipment with which it came in contact. In this experiment the milk contained 3.3 per cent fat and 12.9 per cent solids. It was homogenized at the dairy at 2600 pounds pressure and a temperature of 55° C. (131° F.). The flavor score on delivery in Washington was 23.0 and the viscosity 3.8 centipoises at 20° C. (68° F.). A flavor score of 23 is considered excellent; 21 to 23 is good, and 18 to 21 fair. A flavor score of less than 18 indicates poor or bad milk. Data on flavor indicate the opinion of C. J. Babcock, market milk specialist of the Bureau, without whose assistance it would not have been possible to so accurately judge this property.

Pasteurization of the 5 gallons of homogenized milk was carried out in 3 porcelain-lined containers set in a steam bath, by heating quickly with some agitation to 68° C. (154.4° F.) and maintaining the temperature for 30 minutes. At the end of this period the milk was drawn into 3 glass balloon flasks in 2 to 3 minutes and concentrated under a vacuum of 26 to 27 inches to 6.5 per cent fat and 25.3 per cent solids. The flavor score of the reconstituted milk was 22.5; the criticism, flat. The viscosity of the con-

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TABLE 1-A AND 1-B

Comparison of 3 cold storage temperatures on properties of condensed milk

Weeks in storage	(a) Storage temperature 0° C.				(b) Storage temperature – 7° C.				(c) Storage temperature – 17° C.			
	Flavor	Vis-cosity	Body	Bacteria per cc.	Flavor	Vis-cosity	Body	Bacteria per cc	Flavor	Vis cosity	Body	Bacteria per cc.
1–A. Whole milk homogenized at 2600 pounds pressure and 55° C.; pasteurized by heating to 68° C. and holding for 30 minutes												
		Centipoises				*Centipoises*				*Centipoises*		
1	22.0 Slightly flat	8.6	Good	600	21.5 Flat	8.0	Good	100	22 0 Sl. flat	7.6	Good	900
2	21.5 Flat	8.6	"	20,100	22.0 Sl. flat	8 0	Flakes on bottom of can	200	21 0 Very sl. bitter	7.7	Less flaky than (b)	800
3	19.0 "	9.2	"	600,000	19.0 Sl. oxidized	8.8	Objectionable curd formation	< 100	19.0 Sl. oxidized	8.2	Bottom layer and flakes dissolved on pouring	< 100
4	Sour	9.5	Some fat separation	14,400,000	10.0 Oxidized	8.9	Large lumps curdled		10 0 Oxidized	8.7	Lumpy but better than (b)	
5					15.0 "	11.0	" "	< 100	15.0 "	10.2	" " "	< 100
1–B. Condensed milk homogenized at 2300 pounds pressure and 54° C.; pasteurized by heating to 61° C. and holding for 30 minutes												
1	20.5 Flat	7.9	Good		21.0 Flat	7.4	Some fat separation, sl. flaky		21 0 Flat	7.2	Thin, semisolid layer on bottom of can	
2	19.5 Sl. foreign	8.3	"		21.0 "	8 0	" "		21.0 "	7.2	" " " Better than (b)	
3	18.0 Sl. puckery	9.0	White, watery		20.0 Sl foreign	8 5	Some fat separation, curdy lumps on bottom of can		19 0 Sl. rancid	7 6	Some thickening. Better than (b)	
4	15.0 Rancid	9.2	White, watery, poor		19.5 Sl. bitter	8.6	Curdy lumps larger and firmer than (c)		17 0 Rancid	8.4	Flakes on bottom of can	

centrated product was 6.9 centipoises and the bacterial count 600[1]—a reduction from 3100 per cc. A milk powder-tomato juice medium was used in determining the number of bacteria in the samples. The plates were incubated at 37° C. (98.6° F.).

The condensed milk was cooled to 10° C. (50° F.) and packaged in hermetically-sealed lacquered cans of 140 cc. capacity. The cans were then divided into 3 lots. One lot was stored at 0° C. (32° F.), another at – 7° C. (19.4° F.), and the third at -17° C. (1.4° F.). The effect of the storage temperature on the stability of the condensed milk is recorded in Part 1–A of Table 1–A and 1–B.

Flavor.--The flavor scores of the samples stored at 0° C. decreased at about the same rate during the first 3 weeks as did the scores of those stored at -7° and -17° C. but for a different reason. An increase in the bacterial count was noted at the end of the second week By the end of the fourth week the milk was sour In other experiments quite comparable to this one the rate of increase in the bacterial count was not as rapid and there was evidence that proteolytic organisms predominated. Evidence is ample that bacteria are not quiescent at 0° C. (6) (7).

The flavor scores of the samples stored at -7° C. followed much the same course as did the scores of those stored at -17° C. and for the same reasons. It will be noted that the sample held at -17° C. for 2 weeks was very slightly bitter and was rated 1.0 less than the sample held at -7° C. for the same time. A bitter flavor often precedes an oxidized flavor. Therefore, the evidence here is that, when other conditions are comparable and equally favorable to the development of an oxidized flavor, condensed milk stored at -17° C. develops an oxidized flavor sooner than that held at -7° C. This was corroborated many times in the course of the investigation (See Table 3). If this effect was due to an enzyme which Kende (8) calls oleinase, then we have an unusual example in which an enzyme is apparently more active at -17° C. than at -7° C. In these experiments the rate of freezing was more rapid in the samples stored at -17° C. than in those stored at -7° C., and the latter samples thawed sooner than did those held at the lower temperature when they were placed in the + 6° C. (42.8° F.) incubator where they remained for about 17 hours. But, except for the differences in the rate of freezing and the rate of thawing, the experimental procedure was designed to eliminate variables other than the storage temperature.

The oxidized flavor was less pronounced at 5 weeks than at 4 weeks. It reached a maximum intensity (low numerical score) and then became less pronounced (high numerical score). This will be noted in Tables 3 and 4–A.

[1] Thanks are due F. R. Evans and M. Rogosa for obtaining the bacterial counts.

Viscosity.—The viscosity values in centipoises are relatively small. They were obtained at 20° C. They increased slowly as the samples became older and less rapidly the lower the storage temperature.

Body.—Continued storage in a frozen condition injures the body of condensed milk. If bacterial growth and enzymatic action did not take place at 0° C. it would be better, so far as body is concerned, to preserve homogenized condensed milk without freezing. While the viscosity values obtained subsequent to tempering the samples stored at 0° C. increased faster than did those of the samples stored at –7 and –17° C. subsequent to thawing and tempering, the differences were not sufficient to decrease the quality of the condensed milk from a commercial standpoint.

The body of the samples stored at –17° C. was superior to the body of the samples stored at –7° C. However, in this and other experiments the advantage was not pronounced and, in general, not sufficient to offset the flavor advantages of storage at –7° C.

A batch was made in which metallic contamination was avoided and the heat treatments were relatively mild and in a different sequence.

The milk was produced in the morning and was hand drawn into well-tinned pails. It contained 4.6 per cent fat and 14.3 per cent total solids. The flavor score was 22.5; the criticism, cowy. The viscosity was 3.76 centipoises. In the Bureau's Washington laboratory the milk was drawn directly into glass balloon flasks and sufficient water vaporized to yield milk of 8.0 per cent fat and 26.1 per cent total solids content. This condensed milk was taken to the Beltsville plant, warmed quickly to 54° C. (129.2° F.), and immediately homogenized at 2300 pounds pressure. The condensed and homogenized milk was then returned to Washington and canned in the small tins and pasteurized. The condensed milk was pasteurized in the following manner: The filled and closed cans were placed in metal trays and the trays slid into the places provided in the cage of a pilot sterilizer. The cage rotated in hot water at the rate of 5 revolutions per minute. Under the conditions indicated above it was found that the contents of the cans were at the temperature of the heating medium in 5, 6, 7, and 8 minutes when the water was at 61°, 71°, 76.5°, and 82° C. (141.8°, 159.8°, 169.7° and 179.6° F.), respectively. At the end of the heating period the cans were dumped into cold running water where their contents cooled quickly.

In this experiment the holding temperature was 61° C. and the holding period 30 minutes. The viscosity at the end of this step and subsequent to tempering at 20° C. was found to be 7.2 centipoises. The flavor score of the reconstituted sample was 22.5; the criticism, slightly sweet.

The results are recorded in part 1–B of Table 1–A and 1–B.

Flavor.—Samples stored at 0° C. were inferior in flavor after the first week to those stored at –7° C. and –17° C. After 3 and 4 weeks, samples stored at – 7° C. were superior in flavor to those stored at – 17° C.

The effect of incomplete destruction of the enzyme lipase during processing on the flavor of the stored samples is shown in the results of this experiment. In the presence of lipase that survived the heat incident to processing, the samples, after 2 weeks in storage, became sufficiently hydrolyzed to taste rancid and, unlike the oxidized or tallowy flavor of other batches, the intensity of this flavor continued to increase.

It is not known whether additional experiments would indicate that a rancid flavor becomes more pronounced at -17° C. than at -7° C. This experiment serves to emphasize the importance of destroying lipase before it has had opportunity to adversely affect the fat.

In a commercial operation the properties of the milk and the condensing conditions might be such as to result in a rancid product at the completion of this operation if the lipase in the milk was not destroyed by forewarming.

The lipase should be destroyed before either condensing or homogenizing. According to Dorner and Widmer (9) and Doan (10) this can be accomplished by holding the milk at 60° C. (140° F.) for 10 minutes or by subjecting it to a heat treatment of at least equal enzyme destroying effect. However, if it is assumed that the rancidity noted under the different conditions of the experiments reported here was due to undestroyed lipases, even more severe heat treatments failed to completely destroy the enzyme.

Viscosity.—The increase in viscosity was slower the lower the storage temperature. The initial values were low and some fat separation took place in samples that had been stored at - 7° C.

Body.—In the first 2 weeks the body of the samples stored at 0° C. was satisfactory. There was no fat separation, the product was fluid and did not adhere to the container. But at 3 weeks a change to a whiter, more watery appearance had taken place. This condition was more pronounced at 4 weeks.

The body of the samples stored at -17° C. was slightly superior to the body of the samples stored at -7° C.

EFFECT OF PASTEURIZATION METHODS

It is well known that the effect of high temperatures on the properties of milk and its products is marked. In order to obtain information on this subject, in its relation to frozen condensed milk, experiments were designed to learn whether the heating required to check the development of an oxidized flavor causes a cooked flavor. Another purpose was to study the effect of these heat treatments on the viscosity and the body of the samples.

In the making of evaporated milk the high temperature of sterilization prevents the development of an oxidized flavor and increases the viscosity.

The milk was obtained through the regular channels. It was scored 21.5, cowy, and contained 4.4 per cent fat. The fat content of the condensed material was 8.0 per cent. Immediately after the heat treatments indicated in Table 2 the contents of the cans were cooled quickly to 10° C. in cold running water and then slow frozen in still air at -7° C.

The (a) and (b) samples were inferior to the others because of fat separation and less desirable flavor. The (c) samples had a slightly better flavor but the body was still objectionable. The (d) samples, that is, those pasteurized by revolving them in water at 76.5° C. for 15 minutes had flavor scores and body properties that were, on the whole, superior to those of the other samples in this batch.

It will be noted that the viscosity of the samples increased with an increase in the pasteurization temperature and in the storage period. The homogenization temperature and pressure were favorable to a low viscosity in the samples The homogenization pressure in this experiment was insufficient to prevent separation of the fat during slow freezing and thawing in the media of relatively low viscosity.

While pasteurization by exposing milk in the small cans to a temperature of 76.5° C. for 15 minutes was found not to be sufficient in all cases (see Table 3) to prevent the development of an oxidized flavor during a period of 4 weeks, higher heat treatment caused objectionable body changes during storage Consequently, in so far as the effect of heat during pasteurization is concerned this work indicates that the equivalent of the heat treatment which these (d) samples received ought not to be exceeded in the production of frozen condensed milk that is not to be utilized within a relatively short period. If this heat treatment is exceeded the effect is too detrimental to the body of the product; if it is milder, then oxidative and other changes will be more apt to take place. A balance must be sought in accordance with the experiences of the manufacturer of the product.

None of the samples listed under (a), (b), and (c) were scored "cooked" subsequent to dilution. The (d), (e), and (f) samples tasted slightly cooked or cooked when 1 week old. However, this flavor was not apparent in the samples examined after 2 or more weeks storage.

In Table 3 are presented data on the effect of both pasteurization methods and storage temperatures. A portion of the regular milk supply was used. It contained 3.7 per cent fat and had a flavor score of 23. After condensing to a fat content of 8.0 per cent, the product was heated to 70° C. and homogenized at 1400 pounds pressure. The samples stored at -7° C. solidified in 24 hours; those stored at -17° C. in 6 hours.

From a flavor standpoint the samples stored at -7° C. were preserved better than those held at -17° C.

The creamy body of samples stored at -7° C. was preferable to the curdy condition of the older -17° C. samples. The curdy lumps were apt to be hard and surrounded by a watery phase. In this experiment the body of

TABLE 2

Effect of pasteurization methods on properties of condensed milk stored at −7° C.

Condensed milk homogenized at 1000 pounds pressure and 61° C.

Weeks in storage	(a) Pasteurized by heating for 35 minutes in water at 61° C.			(b) Pasteurized by heating for 15 minutes in water at 71° C.			(c) Pasteurized by heating for 15 minutes in water at 74° C.		
	Flavor	Vis-cosity	Body	Flavor	Vis-cosity	Body	Flavor	Vis-cosity	Body
		Centi-poises			*Centi-poises*			*Centi-poises*	
0	21.5 Flat	6.5	Good	21.5 Flat	6.7	Good	22.0 Sl. flat	6.7	Good
1	21.5 “	7.7	Churning effect objectionable	20.5 Sl. bitter	7.9	Same as (a) but less objectionable	21.5 Flat	8.0	Same as (b) but less objectionable
2	21.0 Trifle bitter, Sl. flat	7.8	“ “ More than last week	21.0 Sl. bitter Tr. flat	7.8	“ “	21.5 “	8.4	“ “
3	19.0 Sl. bitter	8.2	Churned fat on top, curdy lumps in bottom portion	20.0 Flat, Sl. off	9.1	Objectionable fat layer of flaky, churned lumps	20.5 “	9.3	Same as (b)
4	19.5 “ “	9.0	Fat layer on surface. Large lumps in bottom	20.0 “ “	10.1	Same as (a) except fewer lumps in bottom	20.0 “ Sl. off	10.1	Fat separation about same as (b). No lumps on bottom
5	19.0 “ “	10.1	Fat separation. Whiter fluid in bottom	19.0 Sl. bitter	11.3	Fat separation. Cultured buttermilk condition	20.5 Flat	13.1	Fat layer greater than (d). Thickening in bottom portion
	(d) Pasteurized by heating for 15 minutes in water at 76.5° C.			(e) Pasteurized by heating for 15 minutes in water at 79.5° C.			(f) Pasteurized by heating for 15 minutes in water at 82° C.		
0	22.0 Sl. flat	7.2	Good	20.0 Cooked	7.6	Good	19.0 Cooked	8.4	Good
1	21.5 Sl. cooked	9.1	Good. No fat separation	21.5 Sl. cooked	9.8	Good. No fat separation	20.0 “	11.5	Good. No fat separation
2	21.5 Flat	9.5	Sl. flaky. Good consistency	21.5 Flat	10.6	Sl. flaky	21.0 Flat	15.7	Same as (e)
3	21.5 “	11.9	Less fat separation and better than (a), (b), or (c)	21.0 “	15.7	Thickening in can. Creamy buttermilk condition	21.0 “	22.8	No fat separation. Sl. better than (e)
4	21.0 “	11.1	Fat separation less than (e). Thickening on bottom	21.0 “	20.0	Less fat separation but more thickening than (d)	21.0 “	34.5	Fat separation slight. Creamy thickening. Watery in bottom portion
5	21.0 “	14.2	Fat layer larger than (e). Sl. flaky	20.5 “	26.8	Thin fat layer, about same as (f), flaky	20.5 “	38.8	Flaky, watery in bottom portion

TABLE 3

Effect of pasteurization methods and storage temperatures on properties of condensed milk

Condensed milk homogenized at 1400 pounds pressure and 70° C.									
Stored at – 7° C.									
Weeks in storage	(a) pasteurized by heating for 15 minutes in water at 71° C.			(b) Pasteurized by heating for 15 minutes in water at 76.5° C.			(c) Pasteurized by heating for 15 minutes in water at 82° C.		
	Flavor	Viscosity	Body	Flavor	Viscosity	Body	Flavor	Viscosity	Body
		Centipoises			*Centipoises*			*Centipoises*	
0	22.0 Flat	7.8	Good	22.0 Flat	8.8	Good	20.0 Cooked	12.5	Good
1	20.5 Sl. oxidized	7.8	Sl. flaky, otherwise good	20.5 Sl. oxidized	10.1	"	21.0 Sl. cooked	20.1	"
2	15.0 Oxidized	9.1	Sl. fat separation and flakiness	15.0 Oxidized	10.9	No flakes or fat separation. Sl. thickening in bottom portion	21.0 "	21.4	Same as (b)
3	15.0 "	9.2	Sl. fat separation and flakiness. A few lumps	17.0 "	11.6	Sl. flaky	21.0 Flat	25.1	Same as (b) but more viscous
4	18.0 "	10.3	Flaky and curdy particles. No fat separation	19.0 Sl. oxidized	17.2	Creamy. Precipitate on bottom of can	21.0 "	43.0	Thick, creamy, cultured buttermilk body. No fat separation. Objectionable
5	19.0 Sl. oxidized	16.4	Curdling and lumps in bottom of can	18.0 Oxidized	22.5	Creamy but not flaky	20.0 Old	51.3	" " "
Stored at –17° C.									
	(d) Pasteurized as in (a)			(e) Pasteurized as in (b)			(f) Pasteurized as in (c)		
1	15.0 Oxidized	8.8	Sl. flaky, otherwise good	17.0 Oxidized	10.0	Same as (d)	20.5 Cooked	16.1	Same as (d) except sl. flaky in bottom portion
2	15.0 "	9.8	" " No fat separation	18.0 "	10.1	" " "	21.0 Sl. cooked	16.5	Sl. better than (e)
3	15.0 "	9.6	Good, except precipitate on bottom of can	17.0 "	10.7	No flakes, no fat separation. Same as (d)	21.0 "	17.9	Thickening in bottom portion
4	18.0 "	9.9	Lumpy and some thickening in bottom portion	18.0 "	12.0	More thickening in bottom portion than (d). Lumps about same	19.0 Sl. bitter	29.2	Curdy with small flakes
5	17.0 "	13.0	Curdy with watery condition between lumps	19.0 Sl. oxidized	17.9	Inferior to (d)	18.0 Oxidized	43.0	Thicker than (d). Large curdy lumps

the samples stored at -7° C. was superior to that of samples stored at -17° C.

EFFECT OF HOMOGENIZATION

In the making of evaporated milk, homogenization is one of the processing steps. Proper homogenization results in a product of homogenous character with respect to the dispersion of the fat phase; consequently a cream layer usually does not form. Freezing and thawing are injurious to the fat emulsion in both whole and condensed unhomogenized milk. This undesirable effect can be eliminated by dispersion of the fat by means of homogenization. Another but less effective way is to increase the viscosity of the condensed milk through vaporization of water until a solids content of 3 to 4 times that of the whole milk has been attained.

It has been shown by Tracy, Ramsey, and Ruehe (11), Thurston, Brown, and Dustman (12), and Ross (13) that homogenization has a marked effect in reducing or in eliminating the susceptibility of milk to oxidized flavor development.

The effect of homogenization on the properties of frozen condensed milk is shown by data collected in Tables 4–A and 4–B. These 2 batches of milk were obtained at an interval of 1 week and were a part of the general milk supply. The condensed milk contained 8.0 per cent fat.

No explanation is attempted as to why homogenization caused the condensed milk to be more resistant to the changes which result in an oxidized flavor. Ross (13) has a hypothesis for whole milk which is based on the assumption that "oxidized flavor is caused by enzymic action on fat globules. If this is true it seems possible that sufficient homogenization pressures surround the finely divided fat globules with a film. This covering protects the fat globules from enzymic action and thus prevents the development of oxidized flavor." In this connection it is interesting to note that Dorner and Widmer (9) in answering the question: "Why did milk become rancid more rapidly after homogenization?" wrote "The effect of homogenization is to reduce the size of the fat globules. A reduction of the size of globules, while the total volume is kept unchanged, produces an increase of the surface. In fact, the surface of the fat is increased about 10 to 30 times through homogenization. The surface of the fat being greater in homogenized milk, the lipase can attack it better." The important point would seem to be not whether more protein is adsorbed by all the fat, but whether each fat globule is better protected by a thicker or more continuous envelope. With a 10 to 30-fold increase in the surface of the fat globules much more protein would have to be adsorbed on the same volume of fat to obtain a protective envelope of as great thickness as before homogenization.

Greenbank (14) reports a more rapid development of an oxidized flavor in market milk the lower the storage temperature within the range 20° to

TABLE 4-A AND 4-B

Effect of Homogenization Pressure on Properties of Condensed Milk Stored at – 17° C.

4-A. Condensed milk homogenized at 55° C. and pasteurized by heating to 61° C. and holding for 30 minutes

Weeks in storage	(a) Unhomogenized			(b) Homogenization pressure 1000 pounds		
	Flavor	Viscosity	Body	Flavor	Viscosity	Body
		Centipoises			*Centipoises*	
0	20.0 Flat	5.7	Fat separation and flaky	21.5 Flat	6.0	Better than (a)
1	19.0 "	6.0	Objectionable cream layer	19.0 "	6.5	Thin deposit on bottom of can
2	18.0 Oxidized	6.1	" " Bottom portion watery	19.0 Sl. oxidized	6.5	Some fat separation
3	10.0 Rancid	6.5	" "	12.0 Oxidized	7.1	Poor
4	0 "	7.6	" "	17.0 "	8.7	"

4-B. Condensed milk homogenized at 60° C. and pasteurized by heating to 61° C. and holding for 30 minutes

	(a) Unhomogenized			(b) Homogenization pressure 1000 pounds		
0	20.0 Flat	7.3	Fat separation	20.5 Flat	7.7	Good
1	20.5 "	7.4	Objectionable. Flaky cream layer	20.5 "	8.0	Some fat separation. Fair body
2	20.0 "	8.1	" "	20.5 "	8.4	" "
3	19.0 Sl. oxidized	8.6	" "	18.0 Oxidized	8.9	" "
5	17.0 Oxidized	9.1	" "	19.0 Sl. oxidized	11.0	" "

5° C. (41° F.). The results reported above would seem to extend this range to – 17° C. On the other hand, Thurston, Brown, and Dustman (12) show that ". . . freezing and thawing under the conditions obtaining caused the reduction or elimination of the susceptibility of the milks to oxidized flavor development." It should be noted, however, that Thurston *et al.* used unhomogenized whole milk and they report further "whenever an excessive cream plug was observed the milk failed to develop oxidized flavor even with 3.9 parts per million of added copper, but when a less pronounced cream plug was observed some development of oxidized flavor usually was evident."

CONCLUSIONS

1. In making unsweetened condensed milk to be stored at low temperatures, the enzyme lipase should be destroyed by heating prior to other processing steps so as to prevent development of a rancid flavor.

2. Unsterilized, condensed milk can be preserved satisfactorily in the frozen state longer than at 0° C. because bacteria are quiescent at the former temperatures but not at the latter temperature.

TABLE 4-A AND 4-B—(*Continued*)

Effect of Homogenization Pressure on Properties of Condensed Milk Stored at –17° C.

4–A. **Condensed milk homogenized at 55° C. and pasteurized by heating to 61° C. and holding for 30 minutes**

Weeks in storage	(c) Homogenization pressure 2000 pounds			(d) Homogenization pressure 3000 pounds		
	Flavor	Viscosity	Body	Flavor	Viscosity	Body
		Centipoises			*Centipoises*	
0	21.5 Flat	6.7	Good	21.0 Flat	7.7	Good
1	20.0 “	6.8	Better than (a), (b), or (d)	20.0 “	8.2	Thin deposit on bottom of can
2	20.0 “	7.2	Deposit on bottom of can	20.5 “	9.4	Better than (a), (b), or (c)
3	18.0 Bitter	7.5	Flaky condition. Fair body	19.0 Sl. bitter	22.2	Sl. thickening. Poor body
4	12.0 Oxidized	10.4	“ “	17.0 Oxidized	27.7	Flaky, cultured buttermilk body

4–B. **Condensed milk homogenized at 60° C. and pasteurized by heating to 61° C. and holding for 30 minutes**

	(c) Homogenization pressure 1800 to 2200 pounds			(d) Homogenization pressure 3000 to 4000 pounds		
0	21.0 Flat	8.9	Good	21.0 Flat	18.0	Good
1	21.0 “	9.1	“	21.0 “	18.5	Good, viscous
2	21.5 “	10.2	“	21.5 “	20.2	“ “
3	21.0 “	10.7	“	20.0 Off	31.3	Thickening in bottom portion
5	20.0 Old	17.6	Creamy, cultured buttermilk body. Better than (d)	20.0 Old	74.6	Creamy, cultured buttermilk body

3. Other conditions being equal, condensed milk stored at –17° C. is more susceptible to the development of an oxidized, cappy, or tallowy flavor than is condensed milk maintained at –7° C.

4. In order to retard the development of an oxidized flavor in condensed milk that is to be stored for a period of several weeks, the milk should be exposed to as severe a heat treatment as possible without injury to the flavor and body. Under the conditions of these experiments best results were obtained when the condensed milk was heated at 76.5° C. for 8 minutes.

5. Homogenization prevents the formation of an undesirable layer of fat on the surface of condensed milk that has been frozen and aids in retarding changes which are indicated by an oxidized flavor. Pressures

of 2000 to about 3000 pounds per square inch are satisfactory. Higher pressures may be injurious to the body of the product.

6. Market milk may be preserved satisfactorily in the form of homogenized, frozen condensed milk for at least 2 weeks and in favorable circumstances for 4 weeks or longer.

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MILK AND STARTERS FOR BRICK CHEESE

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The irregularity of quality in commercial Brick cheese has been attributed partly to methods of manufacture and to a great extent to the type and quality of starters used. In 1936 a survey was made to study factory methods of making Brick cheese in Wisconsin. This study revealed that a wider variety of methods were used to encourage the development of acidity during the manufacturing process than was originally supposed. It was found that the ordinary *Streptococcus lactis* type of starter was commonly used in the making of cheese from pasteurized milk. Whey starter, however, was commonly used in raw-milk factories. Whey for starter was taken from the vat at the time of dipping, or a portion was saved after skimming. Sometimes the whey was simply set aside in a warm place to sour. Sometimes, especially in the summer, the whey was heated to approximately 150° F. then allowed to cool slowly and was put in a warm place to sour. At other factories the whey was heated to about 180° F. then cooled to 80° F. and inoculated with a small portion of whey saved from the previous day's starter.

METHODS

It was the object of the study to determine the significant types of organisms and their approximate numbers in the starters and to study the methods of carrying the starters in several factories. At the same time observations were made on the types and approximate numbers of significant organisms in the milk supply. Methylene blue tests were made of milk delivered by each patron and in addition observations were made of the quality of the resulting cheese.

Twelve factories located in various parts of the Brick cheese area of Wisconsin were visited during August, 1937. Some of these factories were selected because they were making good cheese, others because they were having difficulty in making cheese of satisfactory quality.

One day was spent at each of the twelve factories in taking samples and making the necessary observations. A sample of starter, samples of milk from individual patrons, and a composite sample of milk representing the supply of the entire factory were obtained on that day. Laboratory work began within an hour or two after the cheese was made.

The starter samples were examined by dilution in litmus milk followed by incubation at 20°, 37° and 48° C.; by Burri slopes; and by microscopic

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examination. The composite samples of milk were treated in the same manner.

Samples of cheese made on the day when the milk and starter were studied were examined for quality and acidity seven or eight days after manufacture. These samples of cheese were obtained by making a second visit to each of the factories.

QUALITY OF MILK

The distribution of patrons according to methylene blue tests of milk delivered at 6 of the 12 factories is shown in Table 1. One might question the meaning of average reduction times which have been calculated for all factories. Average methylene blue reduction times of the patron's milk and estimates of the quality of the cheese are shown in Table 2. Observations in the Swiss and American cheese industries indicate that the chances of producing cheese of high quality are much better when the milk with a long reduction time is delivered to the factories. This relation undoubtedly exists for Brick cheese but is especially obscured in this study by the

TABLE 1

Distribution of numbers of patrons by methylene blue test of milk delivered

Reduction time	Factory number					
	1	5	6	3	10*	2*
Hrs.						
0.3	1	4	....	....	....	....
1	7	4	3	....	....	....
2	8	15	....	2	....	....
3	3	2	....	....	....	....
4	5	4	12	3	....	....
5	8	6	1	10	4	3
6	....	....	4	....	4	2
7	....	....	....	....	....	2
Average time	2.9	2.5	4.0	4.4	5.5	5.9

* Milk delivered twice daily.

TABLE 2

Relation between average methylene blue test and quality of cheese

Factory no.	Reduction time	Quality of cheese	Factory no.	Reduction time	Quality of cheese
	Hrs.			*Hrs.*	
8	1.7	Good*	7	4.4	Fair
5	2.5	Poor	3	4.4	Good
1	2.9	Poor	10†	5.5	Poor
11	3.3	Good	9†	5.9	Fair
4	3.4	Fair	2†	5.9	Good
6	4.0	Poor	12†	6.6	Poor

* Milk was pasteurized before manufacturing.
† Milk was delivered twice daily.

observations made at those factories where milk was delivered twice daily. Standards of quality for such factories, in terms of methylene blue test, must be different from the acceptable standard for other factories. The data of Table 2 simply show that factors other than mere numbers of organisms influence the quality of the resulting cheese.

Important types of organisms and their approximate numbers in milk supplies of poor, fair and good qualities, are shown in Table 3. Data on the milk supply of factory 1 indicate growth of organisms probably induced by lack of cooling or by faulty conditions of production and handling. Observations at factory 7 indicate that the milk is fairly clean but probably lacks cooling. It is interesting to note the relatively small numbers of the *Streptococcus lactis* type of organisms in the milk of factory 2. This factory receives milk from the farmers twice each day and whey is returned to the farms in the milk cans. Since the milk is brought to the factory immediately after it is drawn from the cows there is little or no attempt made to cool it on the farms. It is not surprising, therefore, to find rather large numbers of the *Streptococcus thermophilus* and *Lactobacillus* types of organisms in these milk supplies.

TABLE 3

Approximate numbers of important types of organisms in the milk supplies of three different factories

Types of organisms	Numbers of organisms detected and classified by litmus milk dilution tubes and microscopic examination		
	Factory 1 Poor milk supply	Factory 7 Fair milk supply	Factory 2* Good milk supply
S. lactis	100,000,000	10,000,000	1,000
S. thermophilus	1,000,000	100,000	10,000
Lactobacillus	100,000,000	10,000	100,000
Yeast	1,000,000	none	none
Escherichia-Aerobacter	10,000,000	100	none
Average reduction time	2.9 hours	4.4 hours	5.9 hours

* Milk delivered twice daily.

Methylene blue tests of the factory milk supply and the type of information illustrated in Table 3 might be used to estimate the quality of the milk delivered to each factory. The factors considered in making such an estimate would be the number and types of organisms as indicated by methylene blue tests and dilutions in litmus milk and relative numbers of acid-producing and *Escherichia-Aerobactor* types. Application of this method of estimating the quality of the 12 milk supplies studied places in the "undesirable" or "poor" class some supplies which were satisfactory according to the methylene blue test alone. This estimate of quality seemed to be a little more closely related to the quality of the cheese than the methylene blue test alone. Despite this more complete information upon milk quality which these two examinations afforded, still the prediction of cheese quality from milk quality was unreliable enough to indicate that other important factors were involved.

QUALITY OF STARTERS

The visits to the twelve factories disclosed that nine of the twelve were using pasteurized whey for starter. These whey starters were generally characterized by a rather sweet odor although three of them had a flavor suggesting butyric acid or yeast. These pasteurized-whey starters were made by taking whey from the cheese vat and heating it in a shot gun can to a temperature of about 145° F. The container of hot whey was then set aside to cool to room temperature. Naturally temperature changes were fairly rapid so that the holding time at the effective pasteurizing temperature was sometimes relatively short. The types and numbers of organisms surviving such a heat treatment depend upon the types and numbers of organisms present in the whey before the heating process. Only one operator inoculated the whey after pasteurizing and he simply added a portion of starter saved from a previous batch. If the quality of the milk were very desirable and if acid-producing thermoduric organisms were always present in large numbers this method of carrying starter might be justified. In actual practice, however, it actually propagates the undesirable as well as the desirable types and eventually defective cheese results.

Temperatures of heating curd and whey in the cheese vats ranged between 108° and 118° F. Even the lowest temperature of 108° F. retards the activity of the *Streptococcus lactis* type of organisms but permits the growth of *Streptococcus thermophilus* and the *Lactobacillus* types. It would be expected, therefore, that large numbers of these thermophilic types of organisms should survive the cheese making process and be present in the whey at the time of pasteurizing. Relatively large numbers of such types, therefore, would also be expected to survive the heat treatment and cause acid development in the pasteurized-whey starter. Evidence of the survival of these thermoduric types of acid-producing organisms is presented in Table 4. The approximate numbers of the important types of organisms present in three typical starters are shown in this table. Undesirable as well as desirable types were found. Yeasts were present in four of the whey starters examined. Gas-forming organisms of the *Escherichia-Aerobacter* type were found in three of the nine starters made from whey. The presence of these undesirable types might be attributed to inefficient pasteurization or to contamination after the heat treatment. Although all nine of the factories making pasteurized-whey starters heated the milk in metal containers all of them but one transferred the whey to stone jars in which the whey was held in a warm place while it developed the desired acidity. Such utensils are practically impossible to keep bacteriologically clean and sterilized under ordinary factory conditions.

TABLE 4

Approximate numbers of important types of organisms in three typical starters

Types of organisms	Number of organisms detected and classified by litmus milk dilution tubes and microscopic examination (000—omitted)		
	Factory 5 Poor starter	Factory 4 Fair starter	Factory 3 Good starter
S. lactis	1,000,000,000	1	100,000
S. thermophilus	10,000	1,000,000	1,000,000
Lactobacillus	10,000	1,000,000	none
Yeast	10	10	none
Escherichia-Aerobacter	1,000	none	none

Three factories did not use pasteurized-whey starters. One was using *Streptococcus lactis* in milk, and the other two were using *Lactobacillus bulgaricus* in milk. These starters were made by heating milk in a steam or hot water bath for a period of from one to two hours. The milk was cooled, transferred to a stone jar and inoculated with a mother culture. This mother culture was prepared by simply holding a portion of the bulk culture in a fruit jar until the heated milk was ready for inoculation later in the day. Yeast contaminated two of the three milk starters examined.

Table 5 illustrates the methods of handling them and some of the characteristics of typical starters observed in these factories. The most useful of all of the observations is probably the record of acidity obtained at the time each starter was used in the manufacture of cheese. This acidity is regarded as a general indication of the activity and vitality of the starter. By taking into consideration, therefore, the dominant types of organisms discovered in the starter and the acidity attained at the time the starters are ready for use as well as the characteristic flavor one might estimate the vitality or activity and consequently the usefulness of the starters. Whey starter in which *Streptococcus thermophilus* is the dominant type should attain an acidity of approximately 0.45 per cent, following the usual overnight holding period under factory conditions. Whey starters with the *Lactobacillus* types of organisms predominating should attain an acidity of approximately 0.8 per cent. Starters made with milk and inoculated with *Streptococcus lactis* should have an acidity at the time of using of approxi-

TABLE 5

Propagation and characteristics of typical starters

Factory	5	4	3
Starter type	Heated whey	Heated whey	Heated whey
Inoculation	None	None	Whey starter
Incubation	Boiler room	Boiler room	Make room
Temperature (Approx.)	85° F.	85° F.	75° F.
Acidity	0.41 per cent	0.36 per cent	0.34 per cent
Odor and flavor	Tainted	OK.	OK.

These factories:—(1) Heated whey in shot gun cans to 145° F.; (2) allowed whey to cool in air; (3) placed whey in stone jars to acidify.

mately 0.75 per cent, while an active *Lactobacillus* starter should have an acidity of 1 per cent.

Estimates of the quality of the cheese obtained in these factories were made in terms of three general levels, namely: "poor," "fair" and "good." In Table 6 these general estimates of cheese quality are presented with the corresponding estimates of the quality of the milk and the quality of the starters which were used in making the cheese. Apparently when starter of good quality is used with milk of inferior quality the chances of producing satisfactory cheese seem to be greatly improved. In no instance was a poor starter associated with the manufacture of a good cheese. The quality of the cheese in general was no better than the quality of the starter used in its manufacture.

TABLE 6

Quality of milk, starter and cheese

Factory no.	Quality of milk	Quality of starter	Quality of cheese	
			Estimate	Comments
3	Poor	Good	Good	Long, smooth
8	Poor†	Fair	Good	Long
11	Fair	Fair	Good	Firm, close, sweet‡
2*	Good	Fair	Good	Close, sweet
7	Fair	Good	Fair	Sweet, sl. open, few pin holes
9*	Fair	Fair	Fair	Long, few pin holes, sl. unclean
4	Poor	Fair	Fair	Sweet, pin holes, sl. unclean
10*	Fair	Fair	Poor	Sl. short, pin holes, curdy, unclean
12*	Poor	Fair	Poor	Sl. short, pin holes, sl. unclean
1	Poor	Fair	Poor	Open, pin holes, unclean
5	Poor	Poor	Poor	Open, pin holes, unclean
6	Poor	Poor	Poor	Open, pin holes, unclean

* Milk was delivered twice daily.

† Milk was pasteurized before making into cheese.

‡ Some lots of cheese in this factory were developing openness and splitting at 2 weeks of age. The defect was not found in this cheese at 7 days of age. The starter, however, contained spore-forming organisms which have been found to be associated with this defect in this laboratory.

Our observations seem to indicate, even though the relationship between the quality of the milk and the quality of the resulting cheese is not infallible, that care used in the selection of milk for Brick cheese should result in better cheese. The results of this study indicate definitely that an improved method of propagating starters in cheese factories is needed. The fact that the quality of the starter may tend to limit the quality of the cheese which can be produced from any given milk supply would certainly seem to justify the extra effort and attention necessary to produce starters of the highest quality for the manufacture of Brick cheese.

CONCLUSION

Starter used in the commercial manufacture of Brick cheese could be improved by better methods of propagation. The cheese is usually no better than the quality of the starter used in its manufacture.

ARTIFICIAL INSEMINATION IN DAIRY CATTLE[1]

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The first work in artificial insemination at Minnesota was done with sheep. Technics for semen collection and for the insemination of the ewes were developed. Solutions and methods for the preservation of sperm were also perfected and ewes have been successfully inseminated with sperm six days of age although they have been kept motile for much longer periods of time. We have successfully transported semen back and forth between the Sub-Station at Grand Rapids and University Farm, St. Paul, Minnesota. During the past fall we also successfully shipped semen from Grand Rapids, to the George McKerrow and Sons Farm at Pewaukee, Wisconsin.

During 1935 and 1936, considerable attention has been given to developing technics for the insemination of cattle at the University of Minnesota. During the Spring of 1937 it was thought that enough preliminary work had been completed to give it a field trial. In June of 1937, a small breeding route was organized around the Station at Grand Rapids.

METHODS OF COLLECTING SPERM

Three methods have been used for semen collection:

1. Allowing the bull to serve a cow out of heat. This method has the particular advantage of being very easy and requiring no special equipment except a long glass pipette with a rubber bulb attached.

There are some disadvantages, however: (1) It is often difficult to train an aged bull to serve a cow out of heat. (2) Considerable semen is lost in the folds of the vagina. (3) Semen samples are often contaminated with vaginal discharges. (4) Great care must be used in selecting a host female that is entirely free from any disease or infection that may contaminate the semen.

2. The second method and one that has been used very extensively at Grand Rapids is to massage the ampullae. This method of collection offers some obstacles in that it is sometimes difficult to teach old bulls to react and the technics of successful massage require careful study and considerable practice so that the semen will not be contaminated with urine or carry undue quantities of seminal fluids. The particular advantages are: (1) The semen is obtained easily and quickly after the operator has gained

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experience and the animal has become trained. (2) There is no danger of female contamination: no special equipment is required. (3) What is of most importance, proved bulls that for some reason or other are unable to mount a cow may be kept in service.

3. The third method being used is collection with an artificial vagina. This method was first developed in Russia and has been used with considerable success at Cambridge and other places.

The bull is allowed to mount a blanketed cow and the penis directed into the mouth of the artificial vagina where a normal ejaculation occurs. This method requires some special equipment and considerable care in the use of that equipment. It does, however, provide a way for the complete recovery of a normal ejaculate. Its use undoubtedly results in the best samples. They are uncontaminated by the female; furthermore, urine and excessive quantities of seminal fluids from the male are avoided. The seminal vesicles are often stimulated excessively by the massage method.

It would appear, however, that both of the latter methods will prove very useful to the successful collection of samples on a large scale.

METHOD OF INSEMINATION

The inseminating pipette which we have used is a glass tube about seventeen inches long, $\frac{1}{4}$ inch outside diameter with a $\frac{1}{16}$ inch bore. This is welded at right angles to a tube about five inches long with a $\frac{3}{16}$ inch bore. It is advantageous to have a pocket blown in this piece to serve as a receptacle for the semen. A pyrex glass speculum $1\frac{1}{4}$ inches in diameter and fourteen to sixteen inches long and a flashlight complete the equipment. It is an advantage to the operator to have the speculum made with a light fastened in the end. All the inseminating equipment which we have developed is glass. This provides for easy cleaning and sterilization and low replacement cost. The speculum is used to locate the cervix and the semen is placed directly into the opening; one-half cc. of semen is sufficient to successfully inseminate a cow providing it is placed in the cervix.

When several cows are to be inseminated from one sample or if it is to be held for some time, the semen should be allowed to stand at room temperature for about twenty minutes and then diluted with an equal quantity of diluting solution. The semen is then put into a cooling chamber at 40° to 45° F. until ready for use. After removal from the cooling chamber, it is again allowed to stand at room temperature for 15–20 minutes and then held until it is approximately body temperature.

The normal ejaculate from a bull ranges from 4 to 10 or more cc. Doubling this amount with diluting fluid and using one-half cc. per female will allow inseminating from 16 to 40 females from one service.

The large majority of the cows in our breeding route have been inseminated at the end of oestrum or shortly after. Available data indicate that the bovine ovulates approximately twenty-four hours after passing off heat so that this would seem to be the optimum time for insemination.

DATA ON COWS

A total of 121 cows have been inseminated. Of these, 105 are definitely pregnant, 12 have been inseminated too recently to be certain of pregnancy, and four have been discarded as non-breeders. The miscellanous group as shown in Table 1 has the highest breeding efficiency with a 1:1.32 average.

TABLE 1

Insemination record

	No. of cows inseminated	No. of inseminations	No. of cows pregnant	No. of cows inseminated too recently to be sure of pregnancy	No. of cows failing to conceive	Breeding ratio for cows definitely pregnant
Misc. Group[1]	39	53	34	5	...	1: 1.32
Herd No. I[2]	39	55	37	1	1	1: 1.43
Herd No. II[3]	25	51	21	4	...	1: 2.23
Herd No. III[4]	18	48	13	2	3	1: 3.15
Totals	121	207	105	12	4	1: 1.77[5]

[1] Cows owned in several small herds.

[2] Previous five-year average was 1: 2.

[3] This herd has been in the process of Bang's disease elimination during the entire period of insemination.

[4] This herd has had considerable breeding trouble for several years and artificial insemination was resorted to as an emergency measure.

[5] The four cows that were discarded as non-breeders were inseminated 13 times and are figured in the ratio.

Herd No. I with an efficiency of 1:1.43 shows a decided increase over the previous five year average which was 1:2. Herd No. II has been having a great deal of difficulty with Bang's disease and at least one-third of the cows inseminated were reactors. Herd No. III has had considerable breeding trouble for many years and artificial insemination was resorted to as an emergency measure. Herds II and III do not represent a true picture of the possibilities of artificial insemination but were used in this trial because numbers were needed.

Sixty-four of these cows settled on their first insemination, 27 on their second, and only 13 required more than two inseminations. Eleven of the 13 requiring more than two inseminations were in Herds II and III and three of the four non-breeders were in Herd III.

Artificial insemination is not an emergency measure for getting cows in calf and should not be used for that purpose only. Its successful use will depend on proper supervision and control when it is used in regularly organized breeding groups as a regular procedure. These breeding groups should be under direct supervision of the university of the state in which the work is being done. Specific control measures should provide for the proper sanitation with regard to operators, instruments, and animals. Sanitation cannot be stressed too much if the program is to operate successfully and be a disease control measure. We do not believe it necessary to have a veterinarian specified as the only person qualified to be an operator. Any reliable person drilled in the essentials of sanitation and possessing a knowledge of the reproductive tract should be qualified.

These control measures should also provide for the inspection and certification of any bull to be used in the breeding group.

The successful insemination of a thousand or more cows per year to one sire is not at all beyond definite realization.

Artificial insemination offers to the cattle breeders a tool that gives them an opportunity for the most rapid improvement that has ever been known in cattle breeding. When it is organized as a part of a regular improvement program including butterfat testing, it offers a sure and quick method for proving young sires without special loss or gain to any one breeder and an opportunity to greatly prolong and increase the use of sires that have already proved their merit.

STABILITY OF CAROTENE IN GREEN GRASSES AND ALFALFA STORED AT FIVE DEGREES FAHRENHEIT[1]

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Samples of alfalfa and different varieties of grasses were stored fresh at 5° F. with the assumption that very little or no loss of carotene would occur at this temperature. Several samples were analyzed before putting into cold storage and at the end of 10 months' storage the samples were again analyzed for carotene. There was a definite loss of carotene in the alfalfa samples with little or no loss of carotene in the grass samples. No data could be found in the literature on the stability of carotene in different green forages stored at low temperatures. Data are presented to show that the stability of carotene varies with the type of green forage when stored over a period of time at 5° F.

EXPERIMENTAL PROCEDURE

Alfalfa (*Medicago sativa* L.) and four different varieties of grasses, namely, Kentucky blue (*Poa pratensis* L.), orchard grass (*Dactylis glomerata* L.), meadow fescue (*Festuca elatior* L.) and brome (*Bromus inermis*), were cut green at two weeks' growth at weekly intervals for four weeks during the month of July, immediately packed with dry ice (solid carbon dioxide), sent to the laboratory, and ground green with the aid of dry ice in a Wiley mill. The samples, while still frozen, were thoroughly mixed and a portion of the samples taken for immediate carotene analysis. The other portion of the samples was packed in Mason jars, the lids of which were screwed on loosely to allow the excess CO_2 to escape. The jars were then put back into the original shipping carton to keep out light and immediately put in cold storage at 5° F. while the forage was still frozen. The carotene was determined according to the method described by Guilbert (1) with slight modifications. All calculations are based upon the oven dry basis and the carotene content expressed as milligrams of carotene per 100 grams of forage.

RESULTS AND DISCUSSION

The results of the analyses of the carotene in the forage before and after storage are shown in Table 1. It is to be noted that the loss of carotene in the grasses is negligible. Any difference in the amount of carotene present before and after storage may be due entirely to experimental error in the

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TABLE 1

The carotene content (milligrams per 100 grams of forage on oven dry basis) in forage before and after 10 months' storage at 5 degrees Fahrenheit

	Date collected											
	July 8, 1936			July 15, 1936			July 23, 1936			July 29, 1936		
	Before storage	After storage	Difference	Before storage	After storage	Difference	Before storage	After storage	Difference	Before storage	After storage	Difference
Kentucky blue	19.5	19.5	0	27.0	28.6	+ 0.4	28.5	29.3	+ 0.8	43.0	36.3	− 6.7
Orchard grass	34.4	33.8	− 0.6	41.4	41.0	− 0.4	47.5	44.2	− 3.3	35.3	38.5	+ 3.3
Meadow fescue	28.6	29.5	+ 0.9	54.7	48.1	− 6.6	50.0	45.1	− 4.9	49.3	42.1	− 7.2
Brome	36.2	40.5	+ 3.7	48.6	50.5	+ 1.9	57.5	50.2	− 7.3	43.3	39.2	− 4.1
Alfalfa	21.7	8.01	− 13.7	28.3	7.51	− 20.8	25.0	8.0	− 17.0	15.1	10.0	− 5.1

determination of the carotene. The alfalfa, however, lost, on the average, 62.7 per cent of its initial carotene.

Samples of alfalfa stored under the same conditions the following year showed an average loss of 70 per cent.

These results indicate that a carotene-destroying factor, active at 5° F., is present in the alfalfa and not in the grasses. Since all these samples of forages were stored under the same conditions, the possibility of either light or oxygen being the direct cause for the loss of carotene in alfalfa is apparently eliminated, as one would expect a similar loss of carotene in the grasses. Several samples of dehydrated alfalfa, stored under the same conditions, were analyzed for carotene and showed a loss of only one per cent for the storage period. This difference in the stability of carotene in dehydrated and green alfalfa would indicate that the destructive agent present in green alfalfa is an enzyme.

The results of this experiment indicate that the storage of certain forages at low temperatures does not prevent the loss of carotene, whereas, the storage of other forages studied under similar conditions will prevent this loss. The publication of these results, therefore, should be a warning to other investigators of the danger in storing forages at low temperatures for the preservation of carotene without giving due consideration to the type and condition of forage stored.

SUMMARY

Carotene analyses of green alfalfa and green grasses before and after storage at 5° F. for 10 months show:

1. Alfalfa loses a large percentage of its carotene content.
2. Kentucky blue, orchard grass, meadow fescue and brome lose little or no carotene.

REFERENCE

(1) GUILBERT, H. R. Determination of carotene as a means of estimating the vitamin A value of forage. Jour. Ind. & Eng. Chem., Anal. Ed. 6: 452. 1934.

THE VITAMIN A CONTENT OF DRIED CITRUS PULP

M. C. FUTCH,* L. L. RUSOFF AND R. B. BECKER
Florida Agricultural Experiment Station, Gainesville, Florida

INTRODUCTION

The assay of dried citrus pulp for vitamin A content has been one phase of the investigation of the nutritive properties of citrus by-products at the Florida Agricultural Experiment Station.

Work by Fraps and Triechler (1) indicated that dried orange peel and pulp contained four to six units of vitamin A per gram, and dried grapefruit peel and pulp less than five units per gram, however, these investigators used a limited number of animals and these had high mortality during the assay. Neal (2) observed avitaminosis A in a yearling steer fed a ration of citrus pulp and cottonseed meal (4:1).

EXPERIMENTAL

The procedure outlined in the U.S.P. XI (3) was used in this study except for necessary modification for this type of test material. Normal piebald rats from the stock colony of the Florida Agricultural Experiment Station were used.

The citrus pulp, largely from grapefruit canneries, was dried in a parallel current, direct-heat, stationary drier, agitation of the product being accomplished by rotary paddle wheels. The pulp was ground in a Wiley mill through a one mm. sieve.

On the basis of preliminary assay trials, the final assay groups containing seven rats per group were assigned daily supplement levels of pulp as follows: Group I, 1.0 gram; Group II, 1.25 grams; and Group III, 2.0 grams. The control group was not fed any supplement. The reason for not carrying the assay on higher levels was due to the inability of the animals to consume more than two grams of the supplement daily.

RESULTS AND DISCUSSION

The gain or loss in weight for all the groups during the assay period are presented in Figure 1.

Of all the rats which received the citrus pulp, only five rats on 2.0 grams of pulp daily finished the assay period, making an average gain of 5.0 grams for the period which indicates the presence of some vitamin A in the pulp.

Received for publication July 28, 1938.

* The data upon which this paper is based were taken from a thesis presented by M. C. Futch, in partial fulfillment of the requirements for the degree of M.S., University of Florida.

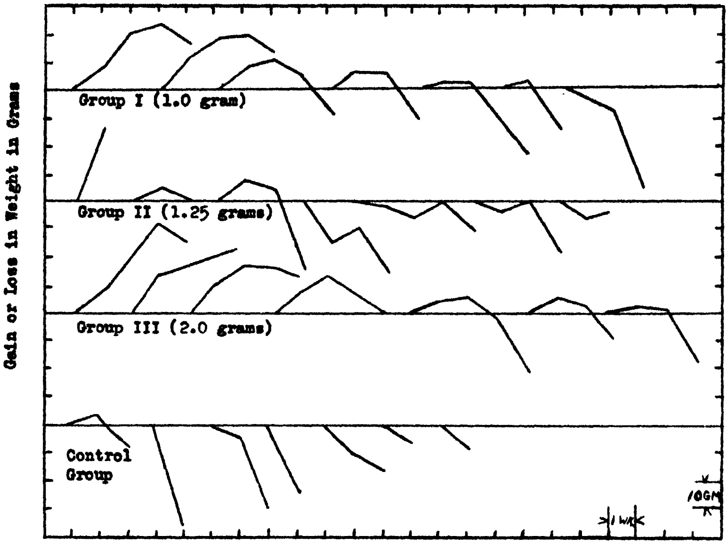

Figure 1.

FIG. 1. Gain or loss in body weight of rats fed different amounts of citrus pulp supplemented to U.S.P. XI vitamin A test ration.

It is evident from the curves that the dried citrus pulp contained less than 0.5 unit of vitamin A (U.S.P. XI) per gram. This low content may be attributed to three causes; low content of vitamin A in the original material, partial destruction of the vitamin in the drying process, or destruction during the period of storage.

SUMMARY

The dried citrus pulp used in this study assayed less than 0.5 U.S.P. XI units per gram of the product.

REFERENCES

(1) FRAPS, G. S., AND TRIECHLER, R. Vitamin content of foods and feeds. Texas Agr. Exp. Sta. Bul. 477. 1933.
(2) NEAL, W. M. Unpublished work.
(3) PHARMACOPOEIA OF THE UNITED STATES OF AMERICA. First supplement, eleventh decennial revision, pages 75–82. 1937. Prepared by the committee of revision and published by the board of trustees. Agent, Mack Printing Company, Easton, Pa.

American Dairy Science Association Announcements

THE STATE COLLEGE OF WASHINGTON AND THE UNIVERSITY OF IDAHO INVITES YOU TO ATTEND THE THIRTY-FOURTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

To the Officers and Members of the
American Dairy Science Association:

It is an honor, as well as a pleasure, for us to know that the American Dairy Science Association has decided to hold its next annual meeting in Pullman, Washington and Moscow, Idaho, June 26–30, 1939. In behalf of the State College of Washington and the University of Idaho we extend a hearty welcome to your Association and hope that a large number of you will avail yourselves of this splendid opportunity of visiting the fine institutions, farms, factories, and scenic wonders of our great Pacific Northwest. We feel certain that you will not only enjoy the traditional western hospitality of our people, but that you will be impressed with the great potentialities of this region with its tremendous natural resources, which are just now beginning to be developed on a magnificent scale. The Grand Coulee Dam, largest structure of its kind in the world, will interest you as will our forests, lakes, streams, mountains and coast.

Dairying is an important industry in this area and our people will be delighted to meet personally the men and women from the length and breadth of this country and others who are the leaders in the scientific advancement of this great industry. We shall spare no effort to make you, your families, and friends comfortable while here. May the 1939 meetings of the American Dairy Science Association surpass all others in attendance, in accomplishments and in personal enjoyment for all of you.

E. O. HOLLAND, *President*	H. C. DALE, *President*
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THIRTY-FOURTH ANNUAL MEETING, STATE COLLEGE OF WASHINGTON, PULLMAN, WASHINGTON, AND UNIVERSITY OF IDAHO, MOSCOW, IDAHO, JUNE *27–30, 1939*

Monday—June 26

1:30 P. M.—General Registration and Room Assignment.

Tuesday—June 27

9:30 A. M.—Dairy Products Judging Conference for Coaches and Instructors.

1:30 P. M.–2:30 P. M.—Opening Session.

2:30 P. M.–4:00 P. M.—General Session (Education).
4:00 P. M.—Meeting of the Board of Directors, and Committee Meetings.
8:00 P. M.—Social Hour.

Wednesday—June 28

8:30 A. M.–9:30 A. M.—Committee Meetings.
Inspection of Extension Exhibits.
9:30 A. M.–12 NOON—Sectional Meetings.
1:30 P. M.–4:00 P. M.—Sectional Meetings.
4:00 P. M.—Committee Meetings.
8:00 P. M.—Reception.

Thursday—June 29

8:30 A. M.–9:30 A. M.—Committee Meetings.
9:30 A. M.–12 NOON—Sectional Meetings.
1:30 P. M.–4:00 P. M.—Sectional Meetings.
4:00 P. M.—Committee Meetings.
6:30 P. M.—Annual Banquet.

Friday—June 30

8:30 A. M.–12 NOON—General Session and General Business Meeting.

CALL FOR TITLES AND ABSTRACTS OF PAPERS

Members of the Association are invited to submit titles and abstracts of papers dealing with original investigations. Those interested in presenting papers should refer to page 789 of the December, 1937, number of the Journal for pertinent information. Time allotted for each paper is limited to 10 minutes. Titles and abstracts of papers must be in the hands of the chairman of the program committee by April 15.

All communications relative to the program should be addressed to Vice-Dean E. V. Ellington, Department of Dairy Husbandry, State College of Washington, Pullman, Washington.

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JOURNAL OF DAIRY SCIENCE

VOLUME XXII MARCH, 1939 NUMBER 3

STUDIES OF LIPASE ACTION. I. LIPASE ACTION IN NORMAL MILK

B. L. HERRINGTON AND VLADIMIR N. KRUKOVSKY
Dept. of Dairy Industry, Cornell University

It is now generally recognized that cows milk contains an enzyme capable of hydrolyzing fat. This enzyme is responsible for the rapid development of rancidity in homogenized raw milk, and the bitter flavor often associated with milk secreted by cows in a late stage of lactation. Milk from cows in an advanced stage of lactation may be abnormal in several respects. This paper deals with lipase action in normal milk which has not been homogenized.

Several changes occur in milk as a result of lipase action. The more obvious are the changes in odor, flavor, and titratable acidity. Most investigations of lipase action in milk have been based upon measurements of the increase in titratable acidity of the milk, but the method has several disadvantages. The end point of the titration is not very distinct, lactic acid fermentation must be prevented, and only a part of the fatty acids liberated by the hydrolysis of fat are water soluble.*

As contrasted with this method, the direct determination of free acid in the fat phase seemed to offer definite advantages. It would not be necessary to correct for acidity due to the protein and salts of milk. Greater quantities of acid might be found in the fat than in the water phase, and the formation of moderate amounts of lactic acid would not interfere. This method was tried and found very satisfactory. With its help, we have been able to detect and measure changes which have been overlooked in the past. After the work was well under way, it was discovered that Paraschtschuck (5) in 1909 and Gould and Trout (4) in 1936 had recommended similar methods for the study of lipase in milk.

THE METHOD

The actual test was performed by churning a half-pint sample of milk,[1]

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We are indebted to the Joseph Willmann Dairy Research Fund for a grant in aid of this investigation.

* Davis (1) has reported that oleic acid is the one set free in greatest amount.

[1] A part of the skimmilk was usually removed first by means of a siphon.

which had been heated previously to destroy the enzyme, transferring the butter to a centrifuge tube, and centrifuging in a heated centrifuge to secure a clear layer of oil. After weighing five grams of the oil into a small flask, 20 ml. of neutral alcohol and 5 drops of 1 per cent phenolphthalein solution were added. The mixture was heated to boiling and titrated with N/20 alkali (NaOH in 50 per cent alcohol). All results in this paper are reported as ml. of N/20 alkali neutralized by 5 grams of fat. This is numerically equivalent to the acid value of Trout, and to the acid degree as recognized by the Association of Official Agricultural Chemists.

THE EXPERIMENTS

As a test of the reproducibility of the titration, one of the writers weighed out a series of samples varying in weight from 0.5 to 6 grams. These samples were titrated by the other without knowledge of the weight of the samples. A linear relation was found between the weight of the sample and the titration. The titration error was approximately ± .03 ml.

After this preliminary test of the method, an experiment was planned to determine normal values for the fat acidity of commercial market milk. Samples of pasteurized milk were obtained from sixty-eight dairy plants distributed over the state of New York. These samples were packed in ice and sent by express to the laboratory, where the acid degree was determined. The results of these analyses are given in Figure 1. The values range from 0.30 to 1.12, but more than half of the values fall between 0.3 and 0.5

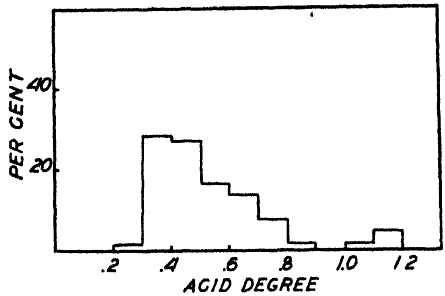

FIG. 1. The distribution of fat acidities in commercial pasteurized milk samples from 68 dairies.

The data of Figure 1 show that the fat in commercial pasteurized milk contains appreciable quantities of free acid, but it is not evidence of enzyme action since the acidity of the fat at the time of milking is unknown. In order to learn the normal values for fresh milk fat, tests were made on the milk of 76 individual cows in the University herd. This herd was made up of Jersey, Guernsey, Brown Swiss, Ayrshire and Holstein cows. The samples represented all stages of lactation. They were pasteurized in glass bottles, and each sample was heated within 5 minutes after the milking was completed. Figure 2 shows the distribution of the values which were obtained. A comparison of these data with that in Figure 1 indicates that lipase must be active in market milk between the time of milking and the time of pasteurization.

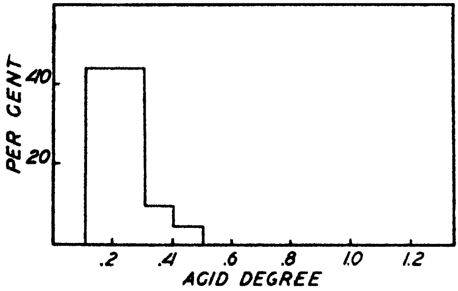

FIG. 2. The distribution of fat acidities in fresh milk samples from 76 individual cows.

These results suggested that when night and morning milk are both delivered to the pasteurizing plant in the morning, it should be possible to distinguish between them on the basis of fat acidity. The night milk should yield higher values because it is older. To test this hypothesis, eight pairs of samples were taken at a local dairy. Each pair consisted of one sample representing all of the night milk and one representing all of the morning milk delivered by one patron. The samples were packed in ice and taken to the laboratory where they were pasteurized at once. Figure 3 shows the

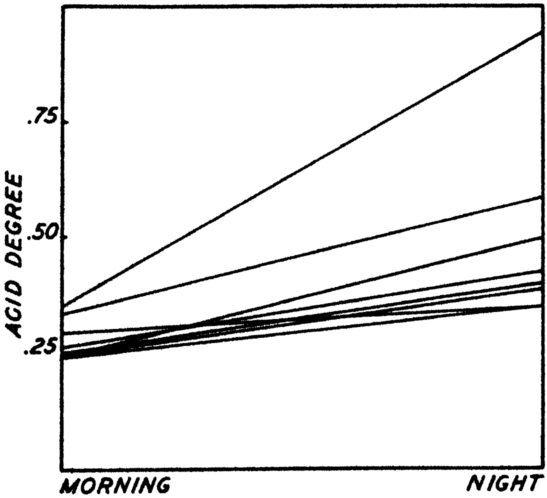

FIG. 3. A comparison of the fat acidities of morning milk and night milk when delivered to a dairy plant in the morning.

relation between the values for the night and morning milk delivered by each patron. In every case, the older milk gave higher values. The great variation in the magnitude of the increase due to aging, indicates that some samples may contain more of the enzyme than others.

These experiments are not conclusive evidence for lipase action in normal milk since the initial value for the evening milk was not determined. In order to secure more conclusive evidence, a series of ten samples were taken from the afternoon milking of the University herd. As soon as the milkers filled a forty-quart can, the can was tagged, and a sample was pasteurized at once. The cans were held in a refrigerated tank of cold water until delivery to the pasteurizing plant the next morning. At that time, another set of samples was taken for comparison with the first set. Figure 4 shows the increase in acidity of the fat between the time of milking and of delivery at the plant. These data are in good agreement with those given in Figure 3 and are considered conclusive evidence of lipase action in normal milk under commercial conditions of handling.

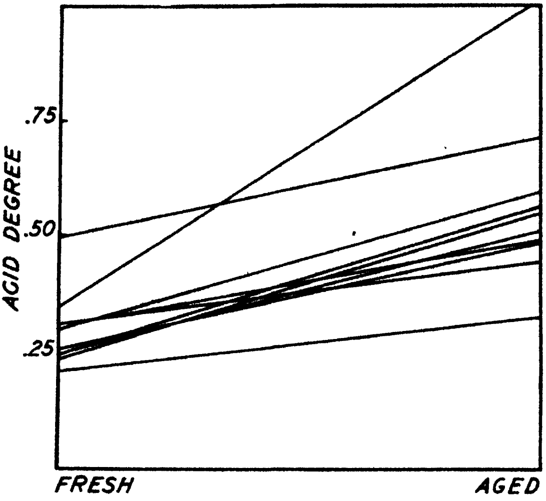

FIG. 4. The increase in fat acidity between time of milking (3 p. m.) and time of delivery at the pasteurizing plant (7 a. m.). Ten cans of milk cooled in cold water at C. U. dairy barn.

The data of Figures 3 and 4 indicate considerable variation between different lots of mixed milk. It seemed likely that even greater variation might be found if a study were made of milk from individual cows. For this purpose, individual samples were taken in the afternoon. Half of each sample was tested at once; the remainder was held in ice water until the next morning. Figure 5 shows the relation between the original acidity and the acidity of the aged sample. The results do indicate a greater variation in enzyme content than was found for mixed milk, though there is surprisingly little variation in the initial values.

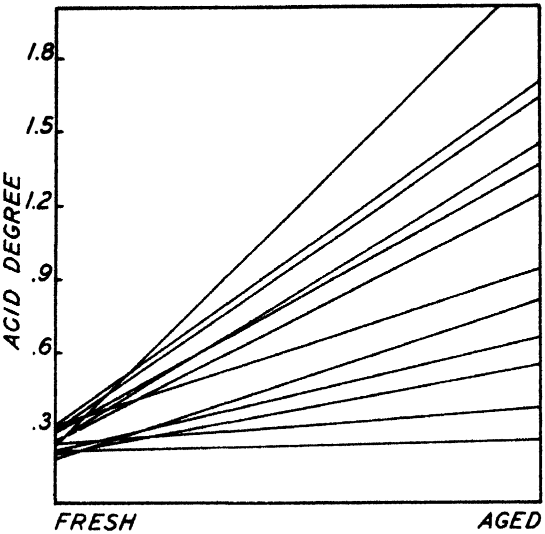

FIG. 5. The increase in fat acidity of milk from individual cows. Milk held 24 hours at 0° C.

These experiments had shown that a definite increase of free acid occurred during a period of 16 hours. In order to learn more about the rate of lipolysis, three series of samples were prepared representing three lots of mixed milk. These were stored in ice water and samples were taken

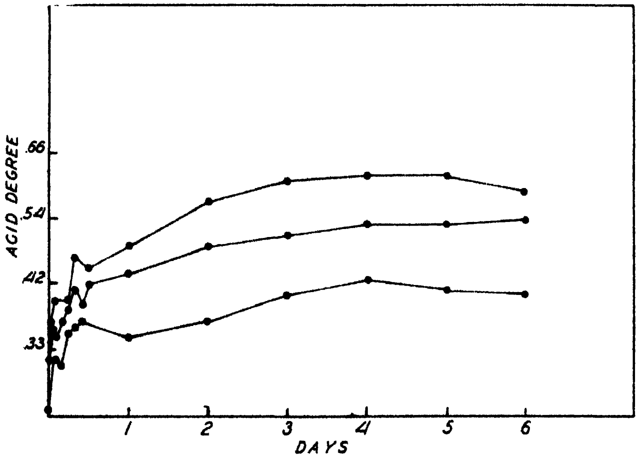

FIG. 6. The change in acid degree of three lots of milk during six days storage in ice water. *The ordinate .33 should read .30.*

from each series after various intervals of time for test. Figure 6 shows that lipolysis was most rapid during the first few hours but that it continued at a slower rate throughout the six days of the experiment.

In order to learn if the rate of cooling were a factor influencing lipase action, three lots of mixed fresh milk were obtained, and each was divided into four parts. One portion was pasteurized at once, without cooling, to serve as a control. A second portion was cooled slowly by placing a half-pint bottle in air at 0° C. A third portion was cooled more rapidly by placing the bottle in ice water. A fourth portion was cooled by means of a small tubular cooler which reduced the temperature from 38° C. to 3.5° C. in less than thirty seconds. These samples were all stored at 0° for 24 hours.

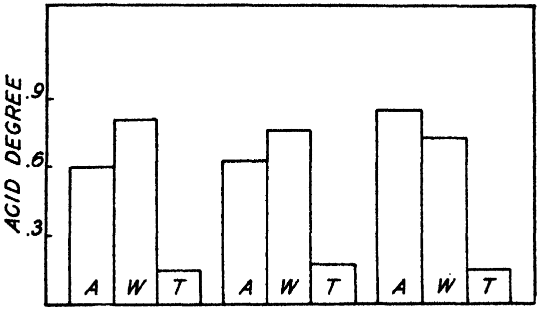

FIG. 7. The effect of rate of cooling upon lipolysis during 24 hours storage at 0° C. three lots of milk, each cooled in air, in water, and, in a tubular cooler. The initial acidities of the milk have been subtracted.

The results of these experiments are shown in Figure 7. Apparently it was of little consequence whether the milk was cooled in air or in water, but very rapid cooling reduced subsequent lipolysis to a marked degree. In connection with this experiment, it should be remembered that, even in air, a half-pint of milk is cooled quite rapidly as compared with the cooling of milk in 40-quart cans. These results suggest that the use of a surface cooler for fresh milk might result in an improvement in quality. This might be of practical value in the production of certified or other raw milk.

It is known that lipase action is accelerated by agitation of the milk (3). In order to determine if the rate of cooling affected the sensitivity of the milk to agitation, portions of the same milks cooled in the preceding experiment were churned raw on the following day. These samples yielded higher values than the preceding set due, in part, to the longer storage period, and in part to the effect of agitation. It is believed that the greater part of the increase was due to agitation since earlier work had indicated that lipolysis during the second day of storage at 0° C. is less than that during the first day. Figure 8 shows the acid degrees which were found. It is apparent that very rapid cooling did not reduce the sensitivity of the milk to agitation.

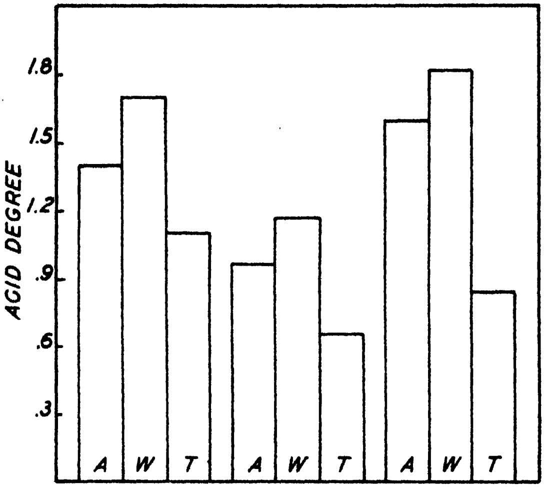

FIG. 8. The effect of rate of cooling upon the acid degree of milk fat churned raw after 48 hours storage at 0° C. Three lots of milk, each cooled in air, in water, and in a tubular cooler. The initial acidities of the milk have been substracted.

Davies (1) has reported that copper reduces lipolysis in milk but he used relatively large amounts of copper (2 and 10 p.p.m.). The effect of smaller amounts of copper upon lipolysis in raw milk was studied by adding copper sulphate to a series of samples. Lipase action at 0° C. was reduced about 20 per cent by 0.2 and 0.4 p.p.m. of copper. Smaller amounts had less effect; larger amounts were not used in this experiment.

Several investigators have used formalin to inhibit bacterial action during the study of lipase action. In general it has been believed that formalin might retard the action of lipase but did not destroy it. Paraschtschuck (5) considered his experiments to be proof that formalin had no action on the lipase of milk. A comparison of his experiment with the following one will disclose the cause of his error.

In order to determine what effect formalin might have upon lipase, a sample of 20 per cent cream was prepared by separating milk at 38° C. within a few minutes after milking. Five samples of 160 ml. each were placed in bottles and formalin was added in increasing amounts of 0, 1, 3, 9, and 27 drops. After careful mixing, the samples were stored in ice water for one week. This experiment was repeated on two subsequent occasions.

Figure 9 shows the increase in acidity which occurred during the storage period. These data show clearly that one drop of formalin in 160 ml. of

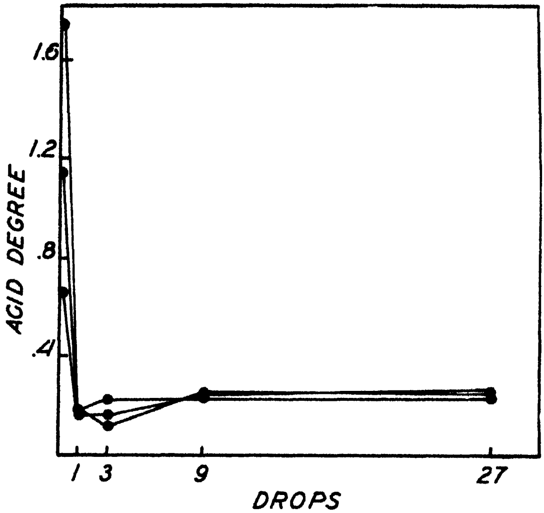

FIG. 9. The effect of 0, 1, 3, 9 and 27 drops of formalin in 160 ml. of milk upon lipolysis during one week at 0° C. Three different lots of milk. The chart shows increase in acidity. The initial values have been subtracted.

20 per cent cream may reduce lipase action to a small fraction of its original value. It also shows that even 27 drops of formalin does not eliminate all of the lipolysis, nor is 27 drops more effective than a single drop. These results are considered as additional evidence for the existence of two lipases in milk (2), one being resistant to formalin, the other being destroyed by it. The criticism that the high value without formaldehyde is due to microorganisms is disproved by the fact that the greater part of the increase in acidity occurs during the first two days of storage.

SUMMARY

By a direct determination of the free acids in the fat phase, it has been shown that lipolysis occurs in practically all samples of raw milk, and that there is an appreciable increase in free fatty acids between the time of milking and the time of delivery to a pasteurizing plant.

There is only a slight variation in the acidity of the fat in the milk of different cows when it is drawn from the udder, but after a few hours there may be wide variations in the acidity.

The rate of lipase action is influenced by the rate of cooling of the milk, being retarded by sufficiently rapid cooling.

Milk contains at least two lipases. One is inhibited completely by very small amounts of formalin. The other is apparently not sensitive to moderate amounts of formaldehyde.

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STUDIES OF LIPASE ACTION. II. THE ACTIVATION OF MILK LIPASE BY TEMPERATURE CHANGES

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In order to account for certain irregularities which were observed in our studies of lipase action in natural raw milk, it seemed necessary to postulate that lipase could be activated by the momentary warming of cold milk. If this were true, it would be of considerable practical importance because raw milk is often allowed to warm slightly without any thought of its possible effect upon lipolysis.

In the following paragraphs, evidence is presented to show that lipolysis in natural raw milk may be greatly stimulated by temperature fluctuations. This phenomenon was studied in some detail, and data are presented to show the importance of the several variables which are involved.[1]

EXPERIMENT 1

The stimulating effect of preheating upon the subsequent rate of lipolysis in milk stored at a low temperature

A 40-quart can of mixed milk from the afternoon milking was cooled over night in water at 3–4° C. After 16.5 hours, the can of milk was warmed by placing it in a tank of water which was heated slowly. Samples were taken from this can as the temperature reached values of 15°, 20°, 25°, 30°, 35°, 40°, 45°, and 50° C. These samples were cooled in water at once, and then stored at 0–5° C. for a period of 48 hours. At the end of that time, a part of each sample was churned raw, and the remainder was churned after pasteurizing to destroy lipase. The acid degrees of these fats were then compared with that of the fat in the fresh milk, with that of milk held the same length of time, but not warmed, and with the value at the end of the heating period before the low temperature storage. This experiment was repeated a week later with another lot of milk.

The data of one experiment is presented in Figure 1. The second gave almost identical results. It is apparent that the warming of cold raw milk greatly accelerates the subsequent lipolysis while the milk is held cold, and that the activation is a maximum when the milk is warmed to approximately 30° C.

EXPERIMENT 2

The influence of the duration of heating upon the subsequent rate of lipolysis

In the experiment just described, the samples were cooled immediately

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We are indebted to the Joseph Willmann Dairy Research Fund for a grant in aid of this investigation.

[1] The methods used in this investigation were those described in the preceding paper.

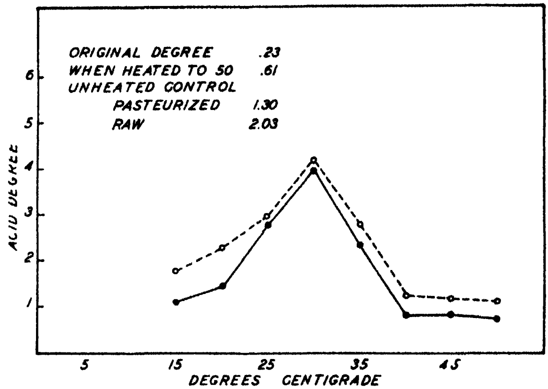

FIG. 1. The relation between the temperature to which milk is preheated, and the acid degree after 48 hours storage at 0–5° C. The values of samples churned raw are indicated by the broken line.

after the desired temperature had been reached. Since it seemed possible that the changes responsible for the activation might not occur instantly, it was thought desirable to hold part of each sample at the specified temperature for a period of time before cooling and storing. Accordingly portions were held for 5, 10, 20, and 30 minutes at each temperature. They were then cooled and held for 48 hours before churning, as described in the preceding experiment.

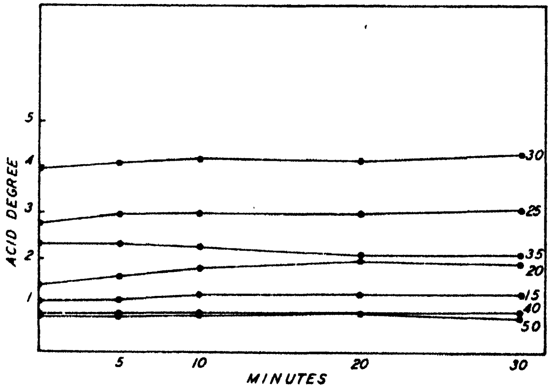

FIG. 2. The relation between the duration of preheating at various temperatures, and the acid degrees after 48 hours storage at 0°–5° C. The 45° line is not shown because of lack of space between the 40° and 50° lines.

The data for the samples of milk pasteurized immediately before churning are shown in Figure 2. The data for the raw samples are omitted to avoid confusion. Both sets of data indicate that the activation, at a given temperature, occurs rapidly and that there is little increase in activation due to holding. In this experiment, the milk was heated approximately 0.37° C. per minute. If it had been heated more rapidly, different results might have been obtained.

EXPERIMENT 3

A comparison of lipolysis in normal and in activated milk

In order to study the comparative rates of lipolysis in normal and in activated milk, a series of identical samples of fresh cold milk was prepared, half of these samples were activated by warming to 30° C. and all were stored in ice water. Bottles were taken at one hour intervals from each series for the determination of fat acidity. This experiment was performed using mixed milk known to show high lipolytic activity and also with mixed milk known to be low in lipase.

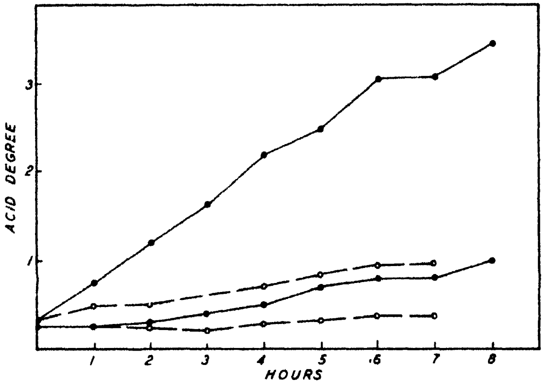

FIG. 3. The comparative rates of lipolysis in normal and activated milk. The activated samples are represented by solid lines. These two lots of milk represent the two extremes which are likely to be found in mixed milk.

The data are presented in Figure 3. It is apparent that the milk fat, activated by warming is attacked very much more rapidly by lipase than that which was not activated. The amount of activation which can be produced by warming is related, however, to the original lipolytic activity of the milk.

These experiments confirmed the original hypothesis that the rate of lipolysis is greatly affected by the previous heat treatment of the milk. In order to learn more about the phenomena involved, a number of additional experiments were performed.

EXPERIMENT 4

The influence of the temperature of pre-cooling upon the degree of activation produced by warming

A can of milk was obtained at milking time and cooled by placing it in ice water. Samples were taken as the temperature reached values of 25°, 20°, 15°, 10°, 5°, and 0° C. These samples were immediately warmed to 30° C. and then placed in a cooler at 0–5° C. After 48 hours storage, a part of each sample was pasteurized and churned. The remainder was churned without pasteurization. This experiment was performed at two different times with different lots of milk. The data of one experiment is shown in Figure 4. The second experiment yielded almost identical curves. It is apparent that the degree of activation possible by warming increases as the pre-cooling is carried to lower temperatures.

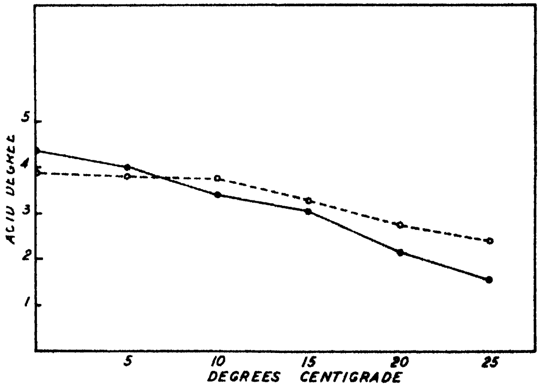

FIG. 4. The relation between the temperature to which milk was pre-cooled, and the acid degree 48 hours after activation. The dotted line represents samples which were churned raw.

It is interesting to note that the milks most highly activated do not respond to shaking during the churning process to so great a degree as those less highly activated. The samples pre-cooled to quite low temperatures actually show less fatty acids when churned raw than when churned after pasteurization. This reversal was noted in a number of cases where the acidity of the fat was quite high. Perhaps this can be explained by assuming that during the agitation, there is a distribution of water soluble acids between the water phase and the surface layer of the fat globule. In the case of pasteurized milk, there is a lower concentration of acid in the surface of the globule because, when the fat was melted, the acid diffused into the center of the fat droplet. Consequently, less acid would be lost to the water phase when that was in equilibrium with the heated fat than when it was in equilibrium with the unheated fat. It is also necessary to assume that lipolysis is retarded by the higher concentration of acids, but this seems fairly well established by a number of experiments.

EXPERIMENT 5

The influence of the duration of pre-cooling upon the rate of lipolysis after activation

In the experiment just described, the samples of milk were rewarmed immediately after they had been cooled to the desired temperature. Since the hardening of fat is a slow process, it seemed desirable to hold portions of the milk at the various temperatures for a period of time before warming in order to determine if prolonged cooling would make possible a greater activation by subsequent warming and cooling. For this purpose, samples were taken from a can of milk during cooling as the temperature reached 25°, 20°, 15°, 10°, 5°, and 0° C. Each sample was stored at its own temperature. A part was activated at once, and other portions were activated after 5, 15, and 45 minutes. The acidity of the fat was then determined after 48 hours storage at 0–5° C.

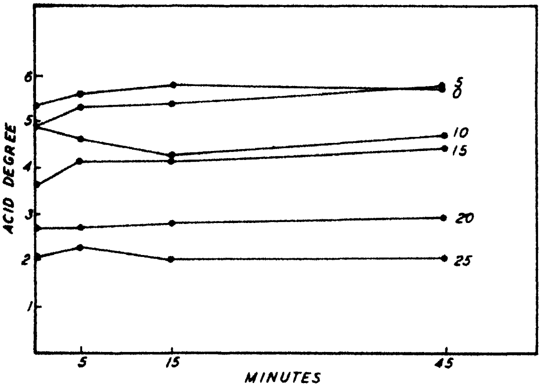

FIG. 5. The relation between the length of time milk is held at various low temperatures before activation, and the acid degree after 48 hours storage of the activated milk at 0–5° C.

The data are shown in Figure 5. Experimental errors are apparent in some of the values, but it seems probable that in the case of normal slow cooling, the temperature to which the milk is cooled is of greater importance than the duration of storage at the low temperature, at least during storage periods of an hour or less.

EXPERIMENT 6

The effect of storage temperature upon the rate of lipolysis in milk activated by warming

A sample of mixed milk was obtained at the barn and cooled to 0° C. in a mixture of ice and salt. It was then warmed to 30° C. in a tank of water at 35° C. and re-cooled. Two samples were taken during the second cooling as the milk reached 25°, 20°, 15°, 10°, 5°, and 0° C. and they were held at the corresponding temperature for periods of 14 and 24 hours, before testing the fat for acidity.

The data are shown in Figure 6. It is clearly evident that cooling after warming is an essential step in the activation process. The full effect of

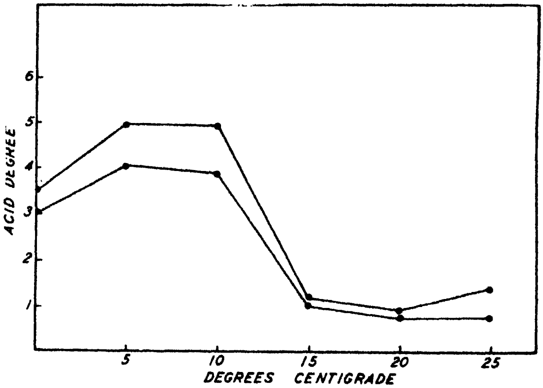

FIG. 6. The relation of the storage temperature of activated milk, and the acid degrees after 14 and 24 hours. The higher values were obtained after 24 hours storage.

warming does not appear unless the milk is subsequently cooled below 15° C.

It must be remembered that two factors are involved in this experiment. It seems reasonable to suppose that the hydrolysis itself (assuming uniform activation) would proceed more rapidly at the higher temperatures. However, the degree of activation might be greater at the lower temperatures, and this would mask the other factor. The experimental data seem to indicate this, since the rate of lipolysis passed through a maximum at a temperature of 5–10° C. However, there is not sufficient data to assert definitely that this is the explanation.

EXPERIMENT 7

The effect of hardness of the fat upon lipase action at a given temperature

It seemed probable that the degree of crystallization of the fat was an important factor governing the rate of lipolysis. The data suggest that, other factors being equal, solid fat is attacked more readily by lipase than liquid fat. This fact could be detected, with certainty, only if both samples of fat were held at the same temperature during storage.

In order to test this possibility, a sample of fresh milk was cooled in ice water to 0° C. Samples were taken at temperatures of 20°, 15°, 10°, 5°, and 0° C. as the milk cooled. The milk was then re-warmed, and a second series of samples was taken at the same temperatures during the heating. These samples were stored at the temperature at which they were taken, and the fat acidity was determined after 20 hours.

It should be noted that the pairs of samples taken at each temperature would differ principally in the degree of hardness of the fat. The fat of the sample which had been warmed from zero to twenty would be nearly solid while that in the sample cooled to 20 would still be liquid. None of these samples were activated by cooling after rewarming.

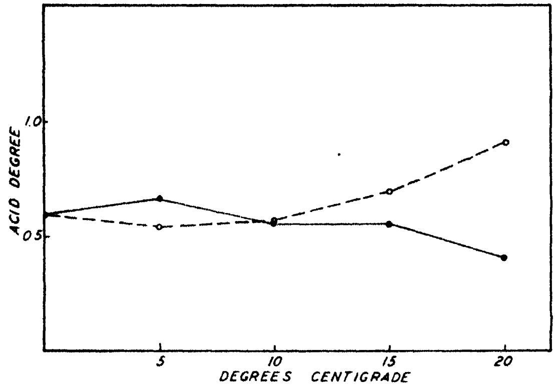

FIG. 7. A comparison of lipolysis of cooled and warmed fat when both are held at the same temperature. Samples represented by the solid line were cooled to the indicated temperature, those on the broken line were warmed from 0° C. to the indicated temperature.

The data are presented in Figure 7. It is apparent that at the higher temperatures the solid fat is attacked more readily than the liquid fat. At the lower temperatures, the reverse is true. The data suggest that there is an optimum degree of solidification for lipase action. If the fat is too thoroughly hardened, lipase action is retarded. This would not be incompatible with the observations made in the previous experiment. It merely indicates another factor which is partially responsible for the observed optimum temperature for lipolysis.

EXPERIMENT 8

The effect of fat hardness upon the degree of activation possible by warming

Since it appeared that these phenomena might be traced in part to the degree of hardness of the fat, it seemed of interest to learn if the degree of hardness of the fat at the time when the milk was re-warmed might be a factor. The data of Figure 5 seem to indicate that it is not, since the activation was not appreciably increased by holding for a longer time. In that experiment it was assumed that the fat held 15 minutes at 10° C., for example, would be more solid than that which was not held. However, in that experiment, the milk was cooled slowly (from 34° C. to 0° C. in 93 minutes) and it is possible that the increased hardening during such a short holding period was negligible.

In order to answer this question more definitely, a lot of fresh milk was cooled to zero and then re-warmed. Samples were taken during the cooling and again during the warming process. Each pair was held at its own temperature for 45 minutes, before activating by warming to 30° C. and cooling to 0° C. These samples were tested for free acid after 48 hours at 0–5° C. The data are presented in Figure 8.

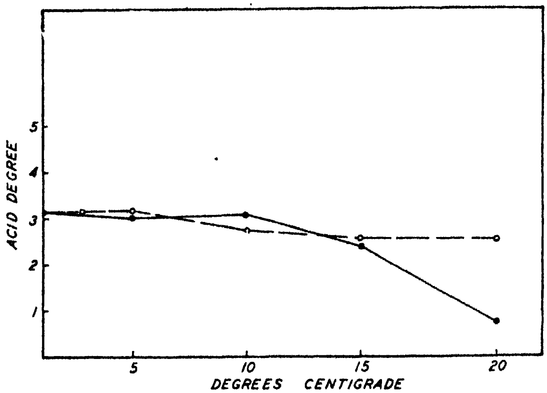

FIG. 8. The effect of fat hardness upon the degree of activation attainable by warming. Before activation, the milk was adjusted to the indicated temperature by cooling (or warming) and holding it for 45 minutes. Storage time 48 hours. Solid line represents cooling.

These results are more difficult to interpret than some of the others. Apparently the effect of low temperature cooling upon subsequent activation by warming is retained for some time even though the milk is stored subsequently at a higher temperature. The data do suggest that there is an optimum degree of pre-cooling for the activation by warming but the difference is too small to be conclusive. In order to learn how long the effect of low temperature cooling might be retained during storage at a higher temperature, this experiment was repeated using a twenty-two hour equilibration period instead of a forty-five minute period. The acid degrees of the different samples were determined at the time of activation and these were subtracted from the final values in order to obtain the increase after activation.

The results of this second experiment were practically the same as the first. The actual values were lower because of the shorter holding period after activation, but the shape of the curves was almost identical with those of Figure 8, showing that the effect of pre-cooling upon subsequent activation was retained, even at 20° C., for at least 22 hours. However, a slight loss of activatibility was apparent at the higher temperatures, amounting to about 10 per cent at 20° C. after 22 hours.

EXPERIMENT 9

The activation and deactivation of deactivated milk

In Experiment 1, Figures 1 and 2, it was shown that heating milk to 30° C. caused an activation of the lipase,[2] while continued heating to higher temperatures caused a deactivation. It seemed of interest to determine if milk could be activated again after it had been deactivated by heating to such a temperature that the lipase was not actually destroyed. For the purpose of this experiment, cold milk reheated to a temperature of 37° C. is termed deactivated; when reheated to only 30° C. it is considered activated. It should be noted that lipase "deactivated" in this way is only partially deactivated. It is still much more active than that in unheated milk.

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[2] In this paper the expression "activation of lipase" is used for convenience only. It seems probable that activation is more dependent upon changes in the state of the fat than upon changes in the lipase.

A sample of fresh cold milk was "deactivated" and stored at 0–5° C. After twenty-one hours, the sample was divided into three portions. One was left as a control, one was deactivated a second time, and the third was activated as previously described. These samples were then stored at 0–5° C. for an additional period of twenty-one hours. The acidity of the fat was determined at the beginning of the experiment, at the time of activation and at the end of the experiment.

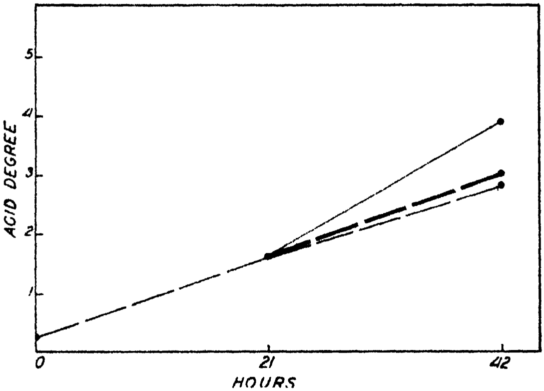

FIG. 9. The effect of activation, or a second deactivation, upon lipolysis in deactivated milk. Activated milk indicated by solid line, double deactivation by a double broken line.

The data are shown in Fig. 9. It is evident that the original sample of "deactivated" milk became rancid quite rapidly. This could be predicted from the results of Experiment One. It is also apparent that such milk can still be activated by proper heat treatment which indicates that the "inactivation" is not due to thermal destruction of the enzyme. The second "deactivation" actually increased the rate of lipolysis though not to so great a degree as by the activation procedure. This is possibly due to the fact that a fresh fat surface is exposed to the action of the enzyme when the fat droplets are melted.

EXPERIMENT 10

The activation and deactivation of activated milk

This experiment was similar to the preceding one, and the same supply of milk was used for both experiments. However, in this experiment, the milk was activated in the beginning instead of being deactivated. Portions were reactivated and deactivated after twenty-one hours, as in the previous experiment.

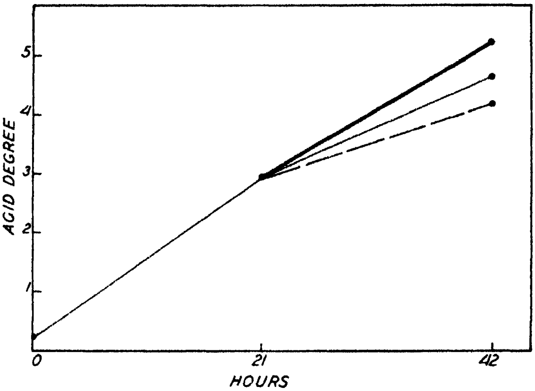

FIG. 10. The effect of deactivation, or a second activation, upon lipolysis in activated milk. Deactivated milk is indicated by a broken line, double activation by two solid lines.

The results of this experiment are presented in Figure 10. It is evident that during the first twenty-one hours, the lipolysis of the activated milk proceeded much more rapidly than in the deactivated milk of the preceding experiment. It is also evident that the "reactivation" was effective in stimulating lipase action. This might be explained by assuming that a fresh fat surface was exposed to the enzyme and water by heating the milk. However, other factors must be involved, because the "deactivated" milk was heated, also, and in that case lipolysis was retarded. Whatever the mechanism may be which is responsible for the phenomenon of activation, it is undoubtedly a reversible process.

EXPERIMENT 11

Showing the possibility of repeated activation of milk lipase

In the preceding experiment, it was shown that lipolysis in milk could be stimulated by a second activation. It seemed of interest to determine how many times the activation might be repeated and still cause a stimulation of lipolysis. In order to secure such information, a series of identical milk samples were prepared. One was used to determine the initial acidity of the fat. The remaining samples were activated at once and again after intervals of 24, 48, 72, 96, and 120 hours. After each interval, one sample was taken from the series for the determination of the fat acidity. This experiment was performed six times, using milk from a different cow for each series.

The data are presented in Figure 11. A few values are missing because it was exceedingly difficult to churn the fat in these highly rancid samples. In a few cases it was impossible.

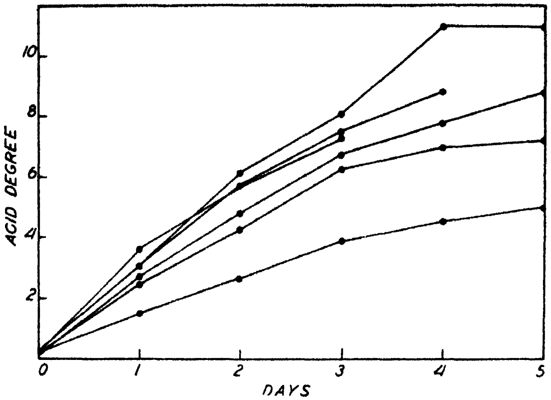

FIG. 11. The progressive increase in acid degree of milks reactivated every twenty-four hours.

In order to interpret the results of this experiment it is necessary to remember that lipolysis normally slows down considerably after the first twelve hours. This was shown in the first paper of this series, and it is shown again in Figure 6 of this paper. In contrast to this normal behavior, these data show that by repeated activation it was possible to secure an almost uniform rate of lipolysis during a period of 96 hours. The data do indicate that there is an upper limit to the acidity obtainable by repeated activation. However, this is of little practical importance because milk would become unmarketable long before such limiting values were even approached.

SUMMARY

Lipolysis in cold milk is accelerated by warming the milk slightly and re-cooling. To secure a maximum of activation, milk should be pre-cooled to 5° C. or lower, re-warmed to 30° C., and then re-cooled below 10° C. The length of time the milk is held at the pre-cooling temperature, or at the activation temperature, is of less importance than the actual temperatures employed, at least in the case of moderate rates of heating and cooling.

The rate of lipolysis seems dependent upon the crystalline state of the fat, and consequently upon the previous temperature history of the milk. Differences in previous temperature history are retained even at 20° C. for at least 22 hours.

Milk which has been activated can be reactivated or deactivated by suitable heat treatment.

These phenomena are of practical importance wherever raw milk is handled. It seems probable that this unrecognized phenomenon of activation has been responsible, in part at least, for many experimental results which have been attributed to other factors.

STUDIES OF LIPASE ACTION. III. LIPASE ACTION IN THE MILK OF INDIVIDUAL COWS

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Most of the data reported in the two preceding papers were based upon a study of mixed milk. However, the first paper did include data concerning the acid degrees of fresh milk from individual cows, and the second contained a comparison of the rates of lipolysis in the milk of a few individuals. This paper contains the results of a more extensive investigation of the milk of individual cows, and of certain factors which might influence the lipase content of milk. The data are published to show the extent of variation, and the complexity of the relations involved. At the present time, no attempt at interpretation can be made.

THE EXPERIMENT

Individual samples were taken from the milk of the 61 cows in the dairy herd of this University at the afternoon milking of either November 27 or December 3. The acid degrees of the fat were determined at the time of milking, and after the milk had been held 24 hours at 0–5° C. A portion of the milk was activated by cooling to 5° C. and rewarming to 30° C., and the acid degree was determined after 24 hours storage at 0–5° C. Another portion was treated with formalin (1 ml. per qt.), activated, and then tested after 24 hours storage at 0–5° C.

From these data, information may be obtained regarding: first, the normal acidity of the fat at the time of milking; second, the variation in the rate of lipolysis of different samples of milk while it is held cold; third, the variation in response to the activation procedure; and fourth, the relative importance of the formalin-sensitive, and the formalin-tolerant lipases in different samples of milk. For comparison with these data, records were kept of the stage of lactation and gestation, and of the yield of milk obtained at the milking from which the samples were taken. (These cows were being milked three times daily at approximately eight-hour intervals.)

All of these data are assembled in Table I. The cows of each breed have been segregated, and arranged according to stage of lactation. To a certain degree, this also arranged them according to stage of pregnancy and milk production.

A study of these data shows that there is little correlation between the stage of lactation and the development of rancidity in milk, with or with-

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TABLE 1

A study of lipase action in the milk of 61 individual cows

Cow no	Weeks of lactation	Pounds of milk	Weeks of gestation	Acid degree			
				Untreated		Activated held 24 hours	Treated with CH_2O activated, held 24 hours
				Fresh	Held 24 hours		
Holsteins							
50	6	27.1		35	90	1.79	1.59
55	6	21.5		25	40	1.41	59
43	7	20.3		33	70	1.60	1.51
42	7	20.7		23	80	2.16	71
48	8	17.1		29	1.46	[illegible]	1.22
36	9	23.5	2	28	59		1.89
56	10	18.6		32	63	1.86	1.10
58	17	10.8	[illegible]	28	1.60	2.50	92
37	18	19.5		25	1.71	2.9	1.42
35	19	20.2		36	70	2.20	82
41	22	18.5	2	32	49	1.81	1.04
44	23	13.3	3	26	4	1.1	79
49	27	17.0	17	27	80	2.21	[illegible]
51	29	15.7	2	30	94	1.71	1.51
59	34	12.9	21	26	86	2.5	1.22
47	35	10.2	20	50	60	1.78	58
38	38	10.2	24	29	1.36	2.39	1.20
52	41	14.4	21	27	1.46	1.89	1.48
40	42	4.9	17	28	27	[illegible]	74
46	4	8.7	23	21	87	2.70	1.07
45	45	15.2	35	19	28	1.7[illegible]	[illegible]
39	49	6.9	25	28	1.03	50	1.[illegible]
Guernseys							
14	3/7			13	20	[illegible]	29
33	3	21.0		21	99	1.38	
22	3	19.7		38	1.89	3.29	
29	4	14.2		20	41	2.1	1.89
12	6	9.0		20	25	2.20	48
17	17	10.6		21	1.20	2.8	2.76
57	18	7.3		32	1.56	2.84	1.78
13	21	8.2	5	20	40	1.85	61
16	32	9.2	21	20	80	68	1.89
1	38	6.8	24	20	31	1.70	27
11	41	5.0	32	30	45	3.75	91
18	51	15.7	3	28	40	00	41
34	60	7.7	8	23	1.48	3.07	1.55
Jerseys							
7	5	7.1		15	55	1.89	59
10	7	15.7		30	1.19	55	2.50
25	14	11.9	3	19	45	2.51	1.76
28	38	8.3	17	20	50	2.88	39
32	9	1.9	14	20	2.48	[illegible].31	1.01
2	40	4.1		20	40	1.59	29
6	41	6.3	25	25	2.06	4.89	1.17
3	44	8.8	15	20	1.54	4.31	1.48
31	44	12.0	2	20	1.30	1.81	2.17
27	47	4.5	24	20	1.79	4.11	1.69
30	52	4.5	16	30	2.21	5.08	2.92
26	60	2.8	4	19	75	2.30	1.26

TABLE 1—(*Continued*)

A study of lipase action in the milk of 61 individual cows

Cow no.	Weeks of lacta-	Pounds of milk	Weeks of gesta-tion	Acid degree			
				Untreated		Activated held 24 hours	Treated with CH_2O activated, held 24 hours
				Fresh	Held 24 hours		
Ayrshire							
20	8	19.7		.20	.39	2.10	1.59
54	15	9.5	5	.16	.56		1.70
19	23	11.7		.19	.30	2.14	1.27
24	43	5.2	9	.13	1.41	3.41	2.52
21	45	7.2	9	.22	1.14	3.40	1.90
15	45	1.6	4	.21	.50	1.96	
53	47	3.9	1	.21	.40	1.30	.43
23	48	3.9	6	.21	.40	2.30	.75
Brown Swiss							
4	2	16.4		.30	.41	1.71	1.33
8	30	8.9	21	.37	.79	2.71	1.74
9	35	11.2	13	.33	.46	3.60	1.09
5	36	10.1	25	.30	2.33	7.73	3.17
60	78	4.7	22	.23	.36	.75	.22

out activation. Similarly, there is no apparent correlation between the stage of gestation, or of milk production with lipase action. There is some evidence of correlation between the rates of lipolysis with and without activation (coefficient of correlation = + .385) but even in this case it is apparent that several factors must be involved and that the relations are not so simple as might be supposed.

These data show that there is a great deal of variation in the nature of the lipases present in different milks, as well as in the apparent amount. For example, the milk of cows 43, 40, 17, and 10 contains principally the

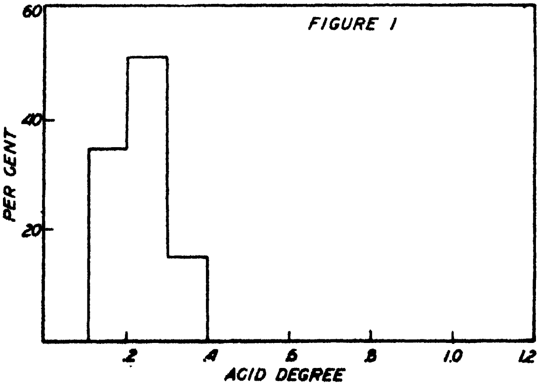

FIG. 1. The distribution of acid degrees in the milk of 61 cows at the time of milking. November and December 1937.

formaldehyde resistant lipase, while that of cows 1, 2, 18, 28, and 60 contains relatively little of this enzyme in comparison with the amount of the formaldehyde sensitive lipase. Most of the samples contain both enzymes, but the proportions in which they occur vary widely.

The fact that in milk there are at least two lipases possessing different properties may be responsible for the fact that so little progress has been made in the study of lipolysis in milk. By studying the behavior of these enzymes separately, it may be possible to explain some of the apparent inconsistencies in our present knowledge.

For comparison with the distribution of acidities of milk fat at the time of milking which was published in the first paper of this series, a similar chart (Figure 1) was prepared from the data of Table 1. It is apparent that the distribution is very similar to that found six months earlier (May). Nearly all of the samples of fresh milk fat which have been examined up to the present time have had acid degrees between 0.15 and 0.35.

SUMMARY

A study was made of lipolysis in the milk of 61 individual cows. A great deal of variation was found in the rates of lipolysis in different samples, and in the response of different samples to activation by warming and cooling. There was no apparent correlation between the rates of lipolysis and the stage of lactation, the stage of gestation, or the milk production of the individual cows.

In some of these samples, the formaldehyde-tolerant lipase predominated; in others, the formaldehyde-sensitive enzyme was more important. Most of the samples contained both enzymes.

STUDIES ON THE ACTIVATED FLAVOR OF MILK

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INTRODUCTION

The specific flavor defect which may be apparent in milk unduly exposed to ultra-violet radiation has been referred to as "activated flavor" by Weckel, *et. al.* (15, 16, 17). The flavor may be described by the terms "burnt," "burnt protein," or "burnt feather." It has also been described as "mushroom flavor" since it is sometimes characteristic of that observed in mushroom soups or broths (17). The flavor may be intensified by heating the irradiated milk to 180° F. or greater followed by cooling. The activated flavor is not to be confused with other flavors such as tallowy, oxidized or cardboard flavors, which may develop in milk following the action of light.

Weckel and Jackson (17) have indicated from their own work and from a comprehensive review of the work of other investigators that the flavor is due to the action of light on the protein fraction of milk. Their review includes the work of Rohr and Schultz (5, 6, 7, 8) who have reported an abnormal flavor in milk which they attributed to the effects of radiation on proteins. They also note the work of Bovie (1), Clark (2), and Stedman and Mendel (9), who have found that the irradiation of various proteins or their solutions leads to the development of "peculiar" or "burnt" odors. The change in character of the absorption spectra of irradiated as compared with non-irradiated solutions of protein, protein derivatives, and amino acids is claimed to be indicative of a significant photochemical effect (17).

On the other hand Doan and Myers (3) indicate that the "burnt flavor" caused by the action of sunlight on milk has its origin in the casein-free and albumin-free serum of the milk. These workers found that a distinct "burnt flavor" developed in this serum when exposed to the sunlight. They call attention to the coincidence between development of this flavor and the fading in color of the serum pigment, lactoflavin.

It has been inferred by Weckel and Jackson (17) that the activated flavor observed in unduly irradiated milk is identical with the burnt flavor observed in milk and skimmilk exposed to sunlight. Hammer and Cordes (4) noted that "off" flavors were developed in certain samples of milk after only 10 minutes exposure to sunlight. Tracy (10) has reported that

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* The authors gratefully acknowledge the assistance given by Mr. H. H. Beck in tasting the samples.

continued exposure of milk to sunlight results in a burnt flavor. The development of such flavor in cottage cheese either within or without glass containers exposed to sunshine has been noted by Tracy and Ramsey (11). Development of the flavor in milk has also been observed by Doan and Myers (3) and by Tracy and Ruehe (12).

Ultra-violet radiation is extensively used for increasing the antirachitic potency of market milk. Since improper use of irradiation may bring about development of the activated flavor, studies were instigated to determine the conditions under which the flavor develops and some of the characteristics of the flavor. A study was likewise made of the burnt flavor which develops in milk on exposure to sunlight, involving a comparison between this flavor and the activated flavor of irradiated milk.

EXPERIMENTAL PROCEDURE

Irradiation of the milk with a strong ultra-violet source was carried out in 200 ml. portions in stainless steel pans 18 cm. square and 3 cm. deep. The pans were placed on a small platform which was given a constant rocking motion by means of an electric motor. The source of ultra-violet light was a Hanovia quartz mercury arc placed directly above the pans at a distance of 76.5 cm. The intensity of the light was approximately 700 microwatts per square centimeter per second at the surface of the film of milk, and was maintained by means of a Hanovia photocell Englehard meter circuit. Subsequent to exposure to radiation the various samples were heated to 180° F. then cooled, since this procedure intensifies the activated flavor. The samples were then examined for intensity of this activated flavor.

The various flavor examinations were made independently by three experienced milk judges.

It was found that with the conditions being used in these experiments an exposure time of seven minutes was sufficient to cause a distinct although not especially strong activated flavor and odor. A strong flavor was undesirable since it was not conducive to accurate distinction between samples.

For the study of the effect of sunlight on the flavor of milk, samples were exposed in 50 ml. portions in stainless steel pans 7.5 cm. square and 2.5 cm. deep. Examination for the flavor was ordinarily made on the unheated milk, but the comparison of these samples with ultra-violet irradiated samples was also made after heating the exposed milk to 180° F. and cooling.

RESULTS OF EXPERIMENTS

A. *The Effect of Heating Milk to Various Temperatures Previous to Irradiation on the Development of Activated Flavor*

Since the heating of milk that has been unduly exposed to ultra-violet radiation intensifies the activated flavor, it was thought desirable to study the effect of heating the milk to various temperatures previous to irradiation on the development of flavor in the subsequently exposed samples.

From Table 1 it is evident that pasteurization of milk does not augment the intensity of the flavor. However, preheating milk to temperatures of 150° F. to 200° F. resulted in a slightly greater intensity of flavor in the milk subsequently exposed to radiation. This difference was not great but, in general, the higher the temperature the more marked was the effect. This indicates that heat has an effect on development of activated flavor even though the heating be done previous to irradiation. However, even with these preheated milks, heating subsequent to exposure to radiation was necessary to bring out the typical flavor under the conditions of these experiments. The samples which had been preheated to 185° F. and 200° F., respectively, possessed an "ozone flavor" without further heating. The ozone flavor is similar to the odor of ozone frequently observed in the vicinity of spark gaps.

TABLE 1

*Effect of temperature to which milk is heated upon its susceptibility to development of activated flavor**

Temperature to which milk is preheated, °F.	Relative flavor intensity
Unheated	½
143.5	½
150	1
165	1½
185	2
200	2

* Milk preheated to temperatures shown, cooled to 70° F., uniformly irradiated, then heated to 180° F., cooled and sampled.

B. *Effect of Homogenization on Activated Flavor*

Due to the fact that homogenized irradiated milk has come into favor, it was thought advisable to study the effect of this double processing on the flavor of milk. An effort was made to determine whether or not homogenization has any effect on the susceptibility of milk to development of activated flavor when unduly exposed to radiation. In general, the results indicate that when irradiation and homogenization are both used in processing milk better results are secured if irradiation precedes homogenization. A but slightly more intense flavor resulted when homogenized milk was irradiated than when milk was irradiated only, or irradiated and then homogenized. Homogenized milk seems to be slightly more susceptible to development of activated flavor when exposed to sunshine than is unhomogenized milk. Perhaps the parent substance or substances which give rise to the flavor may be made more available to, or more susceptible to, the action of the ultra-violet rays and to the sunlight by the homogenization process.

C. *Effect of Altering the "Salt Balance" of Milk upon the Development of Activated Flavor*

Experiments were conducted in which different amounts of various salts were added to milk previous to exposure to radiation to determine whether a disturbance in the normal "salt balance" of the milk might cause an increase or a decrease in intensity of the activated flavor.

When various amounts (0.5 to 5 ml. of molar solutions) of sodium citrate, di-sodium phosphate, calcium acetate, calcium chloride, calcium lactate, or di-basic calcium phosphate were added to 200 ml. portions of milk no definite increase or decrease in flavor intensity was found if the mixtures were then irradiated.

Various sulfur compounds were also added to milk at the rate of 0.25 to 2 ml. of molar solutions per 200 ml. of milk before exposing the milk to radiation. Neither sodium sulfate or sodium thiosulfate had any marked effect on intensity of the activated flavor. No activated odor could be detected in irradiated milk to which had been added sodium sulfite or sodium bi-sulfite. This treatment imparted a strong odor of hydrogen sulfide to the milk and prevented its being tasted. It could not be determined whether the development of the activated odor was being prevented by the compounds added, or whether the odor was merely masked by the hydrogen sulfide.

D. *Effect of Oxidizing Milk on the Stability of the Activated Flavor*

In the course of studies to determine the susceptibility of irradiated milk to development of tallowy or oxidized flavor, it was noticed that samples to which had been added small amounts of copper and which were being held at 40° F., showed a gradual lessening in intensity of activated flavor. Since copper is known to be a catalyst of oxidations, it was thought advisable to investigate the possibility of removing the activated flavor from milk by oxidation.

The addition of a small amount of hydrogen or calcium peroxide either before or after irradiation caused a marked decrease in intensity of flavor. The hydrogen peroxide imparted a somewhat bitter flavor to the milk, while in the calcium peroxide treated samples there was a perceptible lime flavor. It is possible that the activated flavor was being partially masked by these foreign flavors, but the opinion of the judges was that the flavor was actually decreased by addition of the peroxides.

Removal of the activated flavor was accomplished by adding two to three parts per million of copper to the irradiated milk followed by bubbling air through the milk either at room or higher temperature. The best results with this method were secured by holding the milk at 140–145° F. for 30 minutes and bubbling air through it during this interval. Oxidized flavor eventually developed in the milk given this treatment when it was cooled and held at 40° F., but only after 36 to 48 hours.

These experiments in which the activated flavor was removed from milk by oxidation seem to indicate that the compound responsible for the flavor is comparatively easily oxidized, and that oxidation destroys its flavor producing characteristics. However, Swanson and Weckel (14) have found that the addition of oat flour, an anti-oxidant, resulted in decreased susceptibility of the milk to development of activated flavor when compared with untreated samples similarly exposed to radiation. They also observed that milk from cows in early stages of lactation is less susceptible to development of the flavor than is milk from cows that are well advanced in their lactation period.

It is interesting to note that the production of activated flavor by either sunlight or artificial ultra-violet radiation is inhibited when nitrogen is used to replace the usual gas balance existing in milk. Swanson and Weckel (14) removed the air from milk in pyrex flasks by suction. The air was replaced by nitrogen bubbled through the milk. Three evacuations and replacements by nitrogen were made. The periods of the nitrogen treatment were, respectively, five, fifteen and thirty minutes. An atmosphere of nitrogen over the milk was obtained, and the flasks sealed. Flasks of milk not treated by evacuation and nitrogen were similarly sealed.

Exposure of these samples of milk to sunlight on a cloudy day for one hundred forty minutes resulted in the development of a strong activated flavor in the untreated samples of milk, but no activated flavor in the samples of milk treated with nitrogen. When samples of milk prepared in a manner similar to that described above were exposed to radiation from a mercury vapor lamp, inhibition of the development of the activated flavor in the sample treated with nitrogen was again observed, although the inhibition was not as great as in the samples exposed to sunlight.

E. *Effect of Exposure of Solutions of Gelatin and of Egg White to Radiation*

Since it is believed (17) that the protein fraction of milk is the parent substance of the activated flavor, it was thought desirable to study the action of ultra-violet light upon solutions of some proteins other than those derived from milk. Two hundred ml. portions of a one per cent aqueous solution of gelatin were exposed to radiation for various lengths of time. Exposures of five to seven minutes produced a slight activated flavor, and exposures of ten to fifteen minutes produced a characteristic activated flavor when the solutions were subsequently heated to 180° F. and cooled.

The addition of one-third the "white" from one egg to 200 ml. of milk followed by exposure to radiation produced a slightly greater activated odor than was present in control milk to which no egg white had been added. However, the difference was not marked. Half the white of an egg, made up to 200 ml. of solution with water, and given the usual irradiation and heat treatment, resulted in a slight activated odor. (These samples were not tasted for *psychic* reasons, hence the observations reported are of odors only.)

F. *Studies on the Burnt Flavor that Develops in Milk Exposed to the Sunlight*

As stated above, since previous workers (17) have inferred that the activated flavor of irradiated milk is identical with the burnt flavor that develops in milk exposed sufficiently to sunshine, it was considered advisable to compare the two flavors. The flavor studied was the activated, or burnt flavor, and is to be distinguished from the oxidized flavor resulting from the action of light on fats. The oxidized flavor was frequently observed in the course of this work. The flavor of samples of milk exposed to ultra-violet radiation for various lengths of time was compared with that of samples which had been exposed to sunlight for different periods of time. The flavor comparisons were made of the unheated samples and also of samples heated to 180° F. and cooled.

The flavors were found to be strikingly similar, and it was believed by the judges that they were either identical or practically so. Exposures of 50 ml. samples to direct sunlight in 7.5 cm. square stainless steel pans produced the flavor in as little as 10 minutes time. When the milk was heated to 180° F. and cooled previous to tasting, the flavor was detectable in samples which had been exposed only 5 minutes. This is especially interesting in that, as has already been pointed out, the flavor which results from ultra-violet radiation is also enhanced by heating the milk to 180° F. or greater. It is evident from the present work as well as that of previous investigators that ordinary glass as used in milk bottles does not prevent development of the activated flavor due to sunlight. Doan and Myers (3) found that the flavor development was retarded by blue and green colored paper bottles and by blue and green cellophane wrappers on paper bottles. They observed this retarding effect using skimmilk, whole milk, cream and buttermilk.

Various colored glass filters of known wave length transmission values (Corning Glass Company) were used in an attempt to determine the wave length characteristics of radiation which are most effective in causing development of the burnt or activated flavor. These filters along with their transmission ranges are listed in Table 2.

TABLE 2

Relation between development of activated flavor in milk exposed to sunshine and transmission characteristics of glass filters

Glass filter (Corning)		Transmission characteristics (Angstrom units)	Relative intensity of activated flavor
Red Purple Corex A	986	2640–4060	3
Blue Purple Ultra	585	3800–4760	3
Signal Green	440	3820–5960	2
H. R. Lantern Blue	554	3930–5000	2
Noviol Shade C	338	4580–7100	1
Sextant Green	401	4660–5960	1
H. R. Lantern Shade Yellow	349	5460–7100	1
H. R. Signal Red	243	6100–7040	1
Clear Window Glass		3100–	3

The relative amount of flavor development which each of the filters allowed when placed over 50 ml. samples of milk in the 7.5 cm. pans and exposed to sunlight is shown in the table. Four of these filters, Noviol Shade C., Sextant Green, H. R. Lantern Shade Yellow, and H. R. Signal Red, were very effective in inhibiting development of the activated flavor. Exposures of one hour in direct sunlight failed to produce to any significant degree the typical flavor. The other four filters, Red Purple Corex A., Blue Purple Ultra, Signal Green, and H. R. Lantern Blue were less effective in preventing development of the flavor; the first two mentioned being the least effective of all. Each of these four filters allowed development of the flavor within 30 minutes exposure to direct sunlight.

The relative transmission ranges of the four filters which definitely inhibited development of the flavor are 4580–7100 Å, 4660–5960 Å, 5460–7100 Å, and 6100–7040 Å, respectively. The ranges of the less effective filters are 2640–4060 Å, 3800–4760 Å, 3820–5960 Å, and 3930–5000 Å, respectively. The results show that filters which eliminated all wave lengths less than 4600 Å were very effective in preventing development of activated flavor in milk exposed to sunlight. This would indicate that if milk bottles can be devised which will have transmission values comparable to that of the effective filters development of activated flavor in milk exposed to sunlight can be minimized.

The same filters were used with homogenized milk with the same general results except that homogenized milk tends to develop the flavor somewhat more quickly than does regular milk. These experiments were repeated using as filters various types of colored cellophane held between pieces of pane-glass (combined thickness about 0.2 to 0.3 cm.). The transmission characteristics of the cellophanes have been reported by Stebnitz and Sommer (13). Dark green, tango, and dark red were found especially effective, dark blue considerably less effective, while pink, and ordinary parchment were still less effective in inhibiting the development of activated flavor in the milk. The first three colors mentioned prevented development of the flavor in milk exposed to direct sunlight for 30 minutes, while the others allowed development of a definite activated flavor within this 30 minute exposure period. This shows that the darker colors of cellophane are more effective than are the lighter colors in preventing development of activated flavor.

SUMMARY

These studies indicate that heating milk to temperatures above 150° to 160° F. and cooling enhances the activated flavor of the subsequently unduly irradiated milk. When irradiation and homogenization are both used in processing milk better results are secured if irradiation precedes homogenization.

Altering the "salt balance" of the milk by addition of various substances failed to increase or decrease the flavor intensity of the subsequently irradiated milk. Nitrogen and Avenex inhibited development of the flavor.

It was found possible to remove the activated flavor from milk by oxidation. This was best accomplished by adding to the irradiated milk two to three parts per million of copper and then bubbling air through the milk at 140–145° F.

The typical activated odor was produced by irradiation of aqueous solutions of egg white or of gelatin giving additional evidence that the protein fraction of milk is probably the parent substance of the flavor.

The studies indicate that the activated flavor of milk unduly exposed to ultra-violet radiation is identical or practically identical with the burnt flavor which develops in milk exposed to sunlight. Evidence is given that if milk bottles can be devised which will eliminate all wave lengths of light less than 4600 Å they will be effective in inhibiting development of the activated flavor caused by exposure of milk to sunlight.

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DICHLOROFLUORESCEIN AND POTASSIUM CHROMATE AS INDICATORS IN THE ARGENTOMETRIC ESTIMATION OF SALT IN BUTTER

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Potassium chromate has long been used as the indicator in the argentometric estimation of the sodium chloride content of solutions prepared in the analysis of butter according to the method of Kohman (4) as recommended by the Subcommittee on Butter Analysis of the American Dairy Science Association (1). Recently, Bryant (2) has suggested the substitution of the adsorption indicator, dichlorofluorescein, for potassium chromate as the titration indicator. Whereas potassium chromate solutions undergo a color change from yellow to orange-red and thence "brick-red," upon the addition of silver nitrate, salt solutions containing dichlorofluorescein undergo color transition from greenish-yellow fluorescent through whitish opalescent to pink. Under certain conditions the transition of the yellow to orange-red color in the chromate-silver nitrate reaction has not been considered sufficiently critical to permit sharp distinction of the colors. This lack of distinction may easily lead to utilization of an improper end point in the reaction, with accompanying error in the analysis. Hunziker specifically cautions, for example, against excess titration (3). Bryant has observed that the color change from white or whitish appearance to pink state is sudden, and, further, that the results obtained with the fluorescein dye are less (approximately 0.1 per cent salt equivalent) than when potassium chromate is used.

The results obtained using the two indicators, dichlorofluorescein and potassium chromate, when determining the salt content of solutions prepared in the routine analysis of butter received at the Department of Dairy Industry, and in experimental comparisons, are presented to indicate the differences which occur by use of the two methods.

PROCEDURE

In the colorimetric titration of sodium chloride with silver nitrate two factors, at least, may affect the accuracy of the measurement. When the concentration of sodium chloride in the solution is relatively small, the definition of the color end-point may be wanting; when the concentration of silver nitrate in the titrating reagent is relatively great, the ease with which a given color end-point may be developed is limited, and errors acquired in reading the burette have magnified values. The influence of these was con-

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sidered in part by the use of a standard dilution of sodium chloride, *i.e.*, the salt dissolved in 250 ml. of water, the employment of either 17.6 or 25 ml. portions of salt solution for purposes of titration, and silver nitrate solutions of such concentrations that readings as per cent sodium chloride could be obtained either directly from the burette or quickly computed. The following table summarizes the combinations used:

TABLE 1

Summary of procedures used in determining concentration of salt in butter

Method	Quantity of water in which salt from 10 grams of butter was dissolved	Quantity of salt solution used for titration	Silver nitrate per liter of solution	Factor for converting burette readings in mls. to per cent salt
	ml.	*ml.*	*grams*	
A	250	17.6	4.0915	÷ 5
B	250	25.0	5.812	÷ 5
C	250	17.6	20.4	X 1

In the titrations utilizing potassium chromate, the color end point used was that of the first permanent orange-red color. In the titrations utilizing dichlorofluorescein the color end point used was that of the first permanent pink color. A 10 per cent aqueous solution of potassium chromate, and a 0.1 per cent solution in 70 per cent ethyl alcohol of dichlorofluorescein (British Drug Houses Adsorption Indicator No. 22010) were used. Either of two quantities, 2 drops or 0.5 ml., of the indicators were employed, as indicated.

RESULTS

The results of the tests using the two indicators in routine analyses of butter received at the University Creamery are given in Tables 2 and 3.

The results of the titrations for salt in butter using the three methods outlined in Table 1 are given in Table 2. It is evident that in each series where the two indicators were compared, the mean of the titrations using dichlorofluorescein is less than the mean of the titrations using potassium chromate. A lesser difference between the means of two series of titrations using the two indicators occurred when 17.6 ml. of a solution of the salt from ten grams of butter dissolved in 250 ml. was titrated by a solution containing 4.0915 grams silver nitrate per liter.

The variability of the results of titrating a single salt solution repeatedly using first the potassium chromate and then the dichlorofluorescein is shown in Table 3. In this experiment the salt solution was prepared from a single sample of butter and the titrations were made using 17.6 ml. of salt solution

TABLE 2

Percentage of salt in various samples of butter as determined when using 2 drops of indicator solution

Sample No.	Potassium chromate	Dichloro-fluorescein	Difference between indicators	Potassium chromate	Dichloro fluorescein	Difference between indicators	Potassium chromate	Dichloro-fluorescein	Difference between indicators
	Method A			Method B			Method C		
	(a)	(b)	Difference (a)−(b)	(c)	(d)	Difference (c)−(d)	(e)	(f)	Difference (e)−(f)
12	2.54	2.45	.09	2.53	2.45	.08	2.55	2.45	.10
20	1.54	1.46	.08	1.58	1.48	.10	1.60	1.52	.08
51	2.40	2.42	−.02	2.41	2.40	.01	2.50	2.40	.10
89	2.57	2.50	.07	2.54	2.46	.08	2.52	2.50	.02
52	1.76	1.70	.06	1.76	1.68	.08	1.75	1.70	.05
67	2.12	2.08	.04	2.10	2.08	.02	2.13	2.10	.03
15	2.06	2.00	.06	2.05	1.99	.06	2.05	2.00	.05
21	2.16	2.12	.04	2.16	2.10	.06	2.15	2.10	.05
53	1.33	1.30	.03	1.32	1.30	.02	1.31	1.30	.01
97	2.01	1.95	.06	2.00	1.95	.05	2.00	1.95	.05
50	2.53	2.46	.07	2.52	2.46	.06	2.50	2.45	.05
90	1.79	1.76	.03	1.79	1.74	.05	1.80	1.80	.00
14	2.24	2.21	.03	2.24	2.20	.04	2.25	2.25	.00
82	1.22	1.18	.04	1.22	1.16	.06	1.25	1.20	.05
91	2.32	2.30	.02	2.33	2.27	.06	2.30	2.25	.05
92	2.47	2.48	−.01	2.46	2.46	.00	2.45	2.45	.00
16	2.52	2.52	.00	2.49	2.52	−.03	2.50	2.50	.00
0	1.84	1.79	.05	1.83	1.84	−.01	1.85	1.84	.01
Mean	2.079	2.038		2.074	2.030		2.080	2.042	

TABLE 3

Percentage of salt in a sample of butter as determined when using two drops and 0.5 ml. of potassium chromate and dichlorofluorescein indicators

	(Two drops of indicator)		(0.5 ml. indicator)	
	Potassium chromate	Dichloro-fluorescein	Potassium chromate	Dichloro-fluorescein
	2.25	2.20	2.30	2.24
	2.26	2.20	2.30	2.23
	2.28	2.20	2.28	2.20
	2.26	2.20	2.30	2.21
	2.27	2.20	2.32	2.20
	2.24	2.19	2.31	2.21
	2.25	2.20	2.35	2.21
	2.27	2.21	2.28	2.20
	2.23	2.20	2.30	2.19
	2.26	2.20	2.32	2.20
	2.24	2.20	2.30	2.20
	2.28	2.20	2.30	2.20
	2.26	2.18	2.28	2.21
	2.27	2.20	2.32	2.20
	2.28	2.20	2.31	2.19
Mean	2.260	2.198	2.304	2.206
Probable error of mean	± .0027	± .0007	± .0031	± .0022

and silver nitrate solution containing 20.4 grams per liter. The differences between the means shown in this table are statistically significant.

The variability of the results of titrating a single salt solution prepared from recrystallized sodium chloride with silver nitrate solution using first potassium chromate and then the dichlorofluorescein as indicators is shown in Table 4. The differences between the means of the titrations shown in this table are statistically significant. The results of the titrations using dichlorofluorescein were in much better agreement with the calculated percentage sodium chloride of the solution than when potassium chromate was used.

The results of the titrations by a student of a salt solution prepared from butter using first potassium chromate and then dichlorofluorescein as indicators are given in Table 5. The student was asked to make repeat titrations of the solution and was given but two demonstrations of the color end-point of the two indicators. The difference between the means of the two series of titrations in this table also is statistically significant.

Wellings has concluded that the quantity of the adsorption indicator to be used in an argentometric halide titration must lie below the value of 5×10^{-3} mol of indicator per one mol of silver halide, and the concentration as far below this limit as possible, as the color intensity of the titration system is often influenced by the scattering of light caused by the dispersed absorbent (5). In general, it is suggested a concentration of 1×10^{-3} mol

TABLE 4

Percentage of salt in a salt solution as determined when using potassium chromate and dichlorofluorescein indicators (salt solution prepared quantitatively, 3%. NaCl)

	Potassium chromate	Dichlorofluorescein
	(a)	(b)
	3.10	3.00
	3.10	3.00
	3.09	3.01
	3.10	3.01
	3.10	3.00
	3.10	3.00
	3.10	3.01
	3.08	3.00
	3.10	3.00
	3.10	3.01
	3.10	3.01
	3.11	3.02
	3.08	3.00
	3.10	3.01
	3.10	3.01
Mean	3.0973	3.006
Probable error of mean	± .0013	± .0011

of indicator per one mol of precipitate (or equivalent body) may be used as a practical value.

The color change of the reaction mixture may be influenced by the rapidity with which the titration is conducted. The absorption of light by

TABLE 5

Percentage of salt in a salt solution as determined by student using potassium chromate and dichlorofluorescein indicators

	Potassium chromate	Dichlorofluorescein
	2.57	2.56
	2.55	2.54
	2.58	2.58
	2.55	2.52
	2.60	2.51
	2.60	2.57
	2.55	2.55
	2.59	2.57
	2.60	2.58
	2.60	2.55
	2.60	2.56
	2.60	2.54
	2.59	2.59
	2.60	2.52
	2.58	2.51
Mean	2.584	2.550
Probable error of mean	± .0035	± .0048

metal halides is influenced by the absorption of the indicator ions. While these facts may be significant under specific conditions, the results of titrations shown in Table 6 of a salt solution prepared from butter using dichlorofluorescein as the indicator and allowing a lapse of time during the titration indicate that when carried through with reasonable promptness variations of significance may not be anticipated.

TABLE 6

Influence of lapse of time after addition of dichlorofluorescein indicator on results of titration

Sample No.	Time elapsing after indicator plus approximately one-half necessary silver nitrate was added to salt solution (Minutes)	Salt, per cent
1	0	2.95
	5	2.97
	10	2.97
	15	2.95
2	0	2.10
	5	2.10
	10	2.12
	15	2.10
3	0	1.52
	5	1.52
	10	1.54
	20	1.52

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AN INSTANTANEOUS STEAM GENERATOR FOR "STERILIZING" MILK UTENSILS ON THE FARM

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Investigations by Russell (7) Kelly (2) Prucha, Weeter, and Chambers (5) Prucha and Harding (6), and others have shown the importance of sterile utensils in the production of high quality milk. Much of the legislation regarding milk production deals with the sterilization of all utensils with which the milk comes in contact. State laws and municipal ordinances now prescribe the sanitary conditions that must surround the production of retail milk. Bacteriological studies show that contaminated milk deteriorates so quickly as to be unfit for consumption at the time of delivery, or may be the source of infectious diseases.

The study here reported involves a new apparatus for generating steam to sterilize dairy utensils. The principle upon which it operates differs from the steam boiler in that water is vaporized while being pumped through coils heated with a fuel oil burner.

The object of this experiment was to determine the efficiency, cost, and convenience of this steam generator for the production of steam and hot water used in the washing and sterilizing of milk utensils on the dairy farm.

DESCRIPTION OF THE INSTANTANEOUS STEAM GENERATOR[2]

A ¼ H.P. electric motor is directly connected to a triplex pump, one cylinder of which pumps oil to the burner while simultaneously two cylinders of constant capacity pump water or solution to the generator coils (Figure 2). A vaporizing type of burner serves to heat the generating coils. The water in passing through the generator coils is heated to the temperature and pressure necessary to produce a thoroughly conditioned vapor. The manually adjustable burner valve controls the amount of fuel fed to the burner. Adjusting the burner valve to feed more or less fuel to the burner changes the ratio of fuel and water fed to the generator and makes the vapor wetter or drier as desired, regardless of the vapor pressure carried. An automatic pressure control switch shuts off the motor and stops vapor generation when the pressure reaches the desired set point. When the pressure drops slightly the switch closes and vapor generation is resumed. The unit may be operated

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[2] The steam generator used in this experiment was the "Hypressure Jenny," manufactured by the Homestead Valve Manufacturing Company, Coraopolis, Pa.

to produce anything from a steam and water mixture to a superheated steam. It is adjustable from 60 to 150 pounds of steam. This apparatus may also be referred to as a "flash boiler."

DESCRIPTION OF THE UTENSIL AND BOTTLE STERILIZING CHEST

A standard 48 inch by 58½ inch by 28½ inch upright steam chest[3] was used in this experiment. The chest was constructed of 20 gage galvanized iron and had one swinging door. The steam was admitted through a ¾ inch pipe, connected on the inside with two racks of six ¾ inch pipes. One of these racks of pipes was located at the bottom and the other near the middle of the chest. Two pipes on the bottom rack and two on the top rack were perforated with 1/16 inch holes. There were four holes in the pipes near the door and six in the pipes near the back of the chest on both the top and bottom racks. The other four pipes on each rack had no perforations and served only as a heating unit. The steam could be turned off from the perforated pipes without closing the steam to the closed pipes, which kept the utensils hot and thoroughly dried them without taking them out of the chest. Figure 3 shows the position of the pipes in the chest and the location of the steam jets in the pipes.

METHOD

This steam generator was first tested in the laboratory where it was used with the standard steam chest previously described. Cans, pails, strainers, and bottles were contaminated with milk cultures of three different common milk organisms, *i.e.*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*. The milk cultures were prepared using one liter portions of sterilized skimmilk inoculated with pure cultures of these organisms. Approximately 24 hours after inoculation, samples of the cultured milk were taken to determine the number of bacteria per cubic centimeter. Contamination was effected by pouring the suspension of bacteria into the utensil to be sterilized.' Immediately following the contamination, a one-liter portion of sterile saline solution was used to rinse the containers, except the bottles. A total count of this saline solution was made to determine the extent of contamination of these utensils.

After the containers were rinsed with the saline solution they were placed in the sterilizing chest to be steamed. Immediately after the steam was turned off, the door to the sterilizer was opened approximately 1 inch by the use of a notch cut in the latch. This allowed the steam to escape. Five minutes after the steam was shut off, the utensils were removed from the sterilizing chest and rinsed with one liter portion of sterile saline solution.

Milk bottles of quart, pint, and half pint sizes were contaminated and

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[3] Sterilizing chest loaned through the courtesy of the Danzer Metal Works Co., Hagerstown, Maryland.

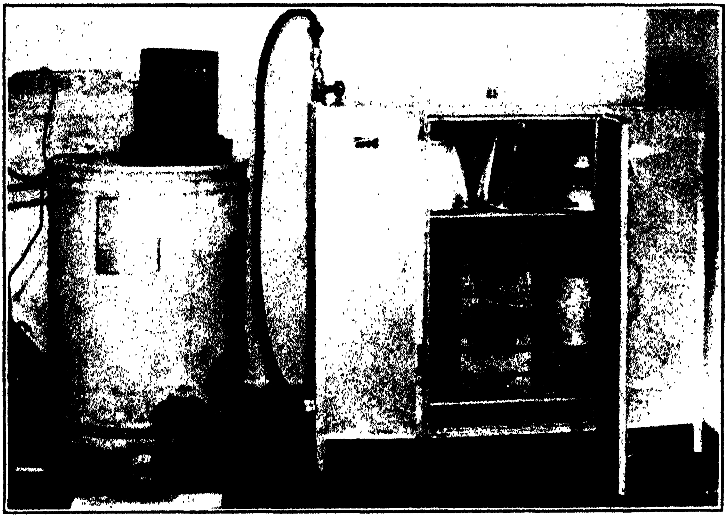

Fig. 1. Instantaneous steam generator and steam chest used in this experiment.

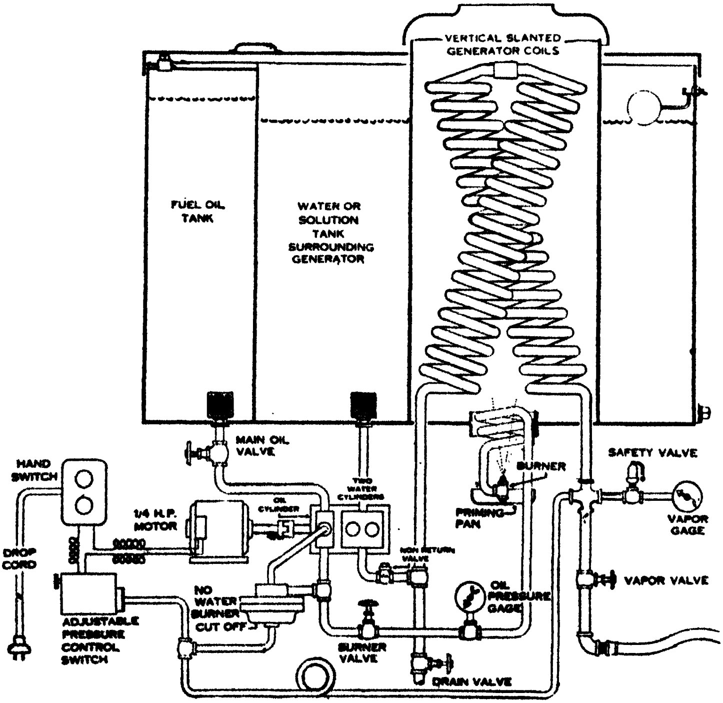

Fig. 2. Diagrammatic sketch of the steam generator indicating the principal parts used in its operation.

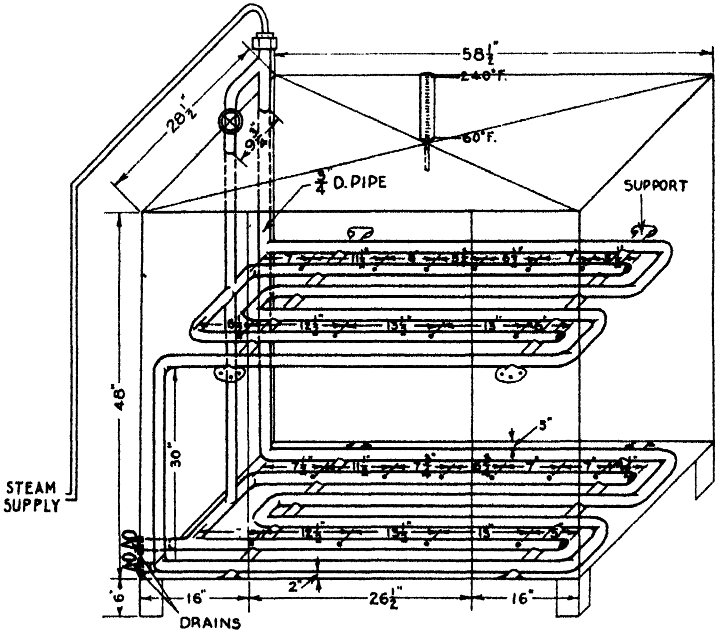

Fig. 3. Diagrammatic sketch of the steam chest indicating the position of the racks and the location of the steam jets in the pipes.

steamed in the same way as the metal containers, except that smaller quantities of sterile water were used in rinsing. Five hundred cubic centimeters were used for quart, 250 cc. for pint and 125 cc. for half pint bottles. The bottles were inverted in the regular milk bottle crates and placed in the steam chest to be sterilized.

The number of bacteria present after steaming was determined by total counts on solid media and the dilution method, employing lactose broth.

Temperature readings were taken during the steaming of the utensils at one minute intervals when the load of utensils in the steam chest had a weight of 225 pounds. The temperature was taken with a tested thermometer located 2 inches from the top of the sterilizing chest and insulated from the metal wall, as shown in Figure 3.

After the completion of the laboratory tests, the steam generator was located on two farms, A and B, both retail milk producers. They continued to use their present equipment, except their steam boilers. Their heating unit was replaced with the described apparatus.

On Farm A, the owner had been using a five H.P. steam boiler which was heated with bituminous coal. The sterilizing apparatus that had been used was a combination brush bottle washer and sterilizer. On this farm approximately 150 quarts of milk were retailed per day.

On Farm B the owner had been using a five H.P. steam boiler, heated with a standard make of fuel oil burner. On this farm a steam chest was used, very similar in design to the one used in the laboratory tests. On this farm, approximately 300 quarts of milk were retailed per day. Steam was used to sterilize equipment three times a day for 20 minutes on farm A, and 30 minutes on farm B, in addition to heating the necessary wash water.

Data on cost of operation was taken for two weeks on each farm, during the months of June and July, 1937.

TABLE 1

Steam sterilization of dairy utensils contaminated with

Time min.	No. of trials	No. bact. per cc. of rinse H_2O before sterilization	No. bact. per cc. of saline solution after sterilization	No. lactose broth tubes showing growth (10 inoculated)
		A *Bacillus subtilis*		
30	4	845,750	0.5	4
20	3	1,200,000	0.94	5
15	4	2,687,500	0.66	6
10	3	790,000	1.75	7.5
5	2	3,900,000	25.7	10
		B *Staphylococcus aureus*		
30	4	3,635,000	0.87	1.5
20	3	1,090,000	0.16	2
15	4	1,835,000	1.08	4.25
10	3	2,240,000	2.0	5
5	2	3,650,000	41.7	10
		C *Escherichia coli*		
30	4	2,035,000	0	0
20	3	5,500,000	0.16	0.2
15	4	3,875,000	0.29	1.5
10	3	3,000,000	1.25	4.5
5	2	4,100,000	23.8	10

RESULTS

It is apparent from the data that this type of steam generator is efficient in generating steam for the destruction of bacteria upon dairy utensils. Table 1 shows the results of steaming metal utensils that had been contaminated with milk cultures of *Escherichia coli, Staphylococcus aureus,* or *Bacillus subtilis.* Table 2 shows the results of steaming similarly contaminated milk bottles. The data in the two cases are much alike.

The results indicate that a 15-minute treatment under the given load is sufficient to render the utensils practically free from bacteria. Longer periods of steaming do not appear to have any advantage in the sterilizing operation. Passon (4) found that "if the steam is evenly distributed in the cabinet, and

TABLE 2

Steam sterilization of milk bottles contaminated with

Time Min.	No. of Trials	Quarts			Pints			Half Pints		
		No. Bact. per cc. of rinse H_2O before sterilization	No. Bact. per cc. of saline sol. after sterilization	No. Lactose broth tubes showing growth (5 inoculated)	No. Bact. per cc. of rinse H_2O before sterilization	No. Bact. per cc. of saline sol. after sterilization	No. Lactose broth tubes showing growth (5 inoculated)	No. Bact. per cc. of rinse H_2O before sterilization	No. Bact. per cc. of saline sol. after sterilization	No. Lactose broth tubes showing growth (5 inoculated)
					A *Bacillus subtilis*					
20	3	2,200,000	.16	2.5	2,150,000	.33	2.5	2,200,000	.5	1.5
15	3	2,750,000	1.0	1.5	1,500,000	.83	5	580,000	.5	2.5
10	3	3,700,000	1.16	4.5	2,600,000	.55	4	5,350,000	.5	5
5	2	2,850,000	37.5	5	2,850,000	26.5	5	2,100,000	40.0	5
					B *Staphylococcus aureus*					
20	3	12,500,000	.16	.5	1,650,000	0	0	1,800,000	.16	.5
15	3	260,000	.16	0	3,950,000	.8	2.5	4,200,000	.50	1.0
10	3	750,000	.85	1.5	850,000	0	0	370,000	0	0
5	2	2,800,000	72.0	5	1,500,000	84.5	5	2,150,000	130	5
					C *Escherichia coli*					
20	3	1,050,000	0	0	1,700,000	.5	.5	4,200,000	.16	1
15	3	1,250,000	0	0	7,850,000	1.15	1.5	5,750,000	.5	.5
10	3	3,000,000	0	0	2,300,000	0	0	1,700,000	.66	0
5	2	9,200,000	20.5	5	8,900,000	33.5	5	100,000	32.0	4.5

it is ascertained from a reliable thermometer placed in the top of it that a temperature of 200° F. or over has been maintained for 5 minutes or more, it is certain that practical sterilization of utensils has been obtained."

The data show that with both metal containers and bottles, the least heat-resistant organism, *Escherichia coli* (Tables 1C, and 2C) is killed in the 10 minute period of treatment. In the case of the more heat resistant type, *Staphylococcus aureus* (Tables 1B, and 2B), the 15 minute treatment is significantly better than the 10 minute. With the most heat-resistant, spore forming type, *Bacillus subtilis* (Table 1A, 2A), the results are inconsistent, even in the longer periods of treatment. Nicholas, Sperry and Tomey (3) found that the temperature of the utensils for the destruction of *Bacillus subtilis* is 325° F.

Figure 4 shows the range of temperature in the steam chest during various lengths of steaming when the total weight of the load to be sterilized was 225 pounds. These temperatures were taken with a tested thermometer located at the top of the sterilizer. Farrell (1) found the temperature variation within a steam cabinet is not very great and that the temperature of the utensils follows very closely the temperature of the sterilizer.

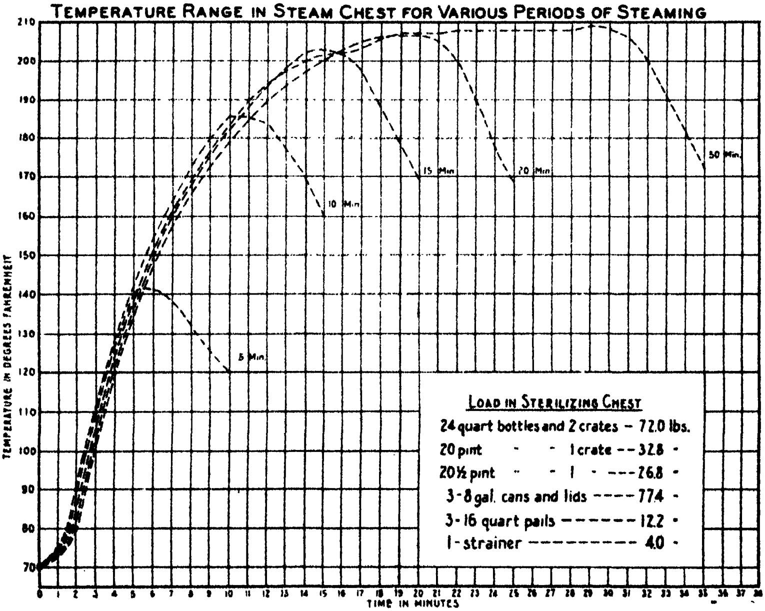

FIG. 4.

The curves show that the temperature in the steam chest rises rapidly when the steam is first turned on, and that the rate of heating decreases as the temperature approaches the maximum of 212° F. The temperature also falls

TABLE 3

Data on sterilization of dairy equipment, including bottles, milking machines, cans, pails, and strainer

	FARM A			*FARM B*	
	Size, 150 qts. per day			Size, 300 qts. per day Steam chest—5′ × 6′ × 3′	
	Hypressure JENNY	Oil steam boiler	Coal steam boiler	Hypressure JENNY	Oil steam boiler
Time required to obtain steam pressure	3–5 min.	40–60 min.	60–90 min.	3–5 min.	40–60 min.
Steam pressure carried	75#	40–50#	40–50#	75#	50#
Fuel oil used (2 weeks)	38 gal.	66 gal.		70 gal.	165 gal.
Coal			¾ ton		
Cost of fuel (2 weeks)	$2.85	$4.95	$3.36	$5.25	$12.37
Size of boiler	Equiv. 5 H.P.	5 H.P.	5 H.P.	Equiv. 5 H.P.	5 H.P.
Time required to reach 180° F. in steam chest	(no steam chest used)			15 min.	15 min.

more rapidly during the first part of the cooling, with the exception of the first minute, in which there is very little change in temperature. The rate of cooling decreases as the temperature approaches that of the room.

The utensils were dry when removed from the steam chest, providing they were not removed immediately after the steam was turned off and the steam pressure was maintained in the closed pipes for 5 minutes to serve as a drier.

The machine was found simple and convenient to operate. It was put into operation by pressing an electric switch, and lighting the burner. From this time on it operated independently. If the steam was turned off the automatic switch stopped the machine at the set pressure, and as soon as the valve was opened, operation was automatically resumed.

In Table 3 are shown the data on the sterilization of dairy equipment in farms A and B, where the apparatus was used under actual operating conditions. Under these conditions, the apparatus was compared with the oil steam boiler and the coal steam boiler and data obtained on the time required to obtain steam pressure, steam pressure carried, size of the boiler, time required to reach 180° F. in the steam chest and the comparative costs.

CONCLUSIONS

An instantaneous steam generator is especially advantageous when steam is needed frequently for short periods of time.

The advantages of the described instantaneous steam generator to generate steam for the sterilization of milk utensils and bottles as shown in this experiment are as follows:

1. It generates sufficient steam to render the utensils practically free from bacteria in 15 minutes of operation.
2. It requires only 3 to 5 minutes to generate steam pressure of 75 pounds.

3. It is small and more compact than the steam boiler.

4. It requires a minimum amount of labor to operate.

5. The apparatus is easy to operate; it starts by pushing an electric switch and lighting the burner and runs independently after setting the pressure.

6. It produces a minimum amount of dust, dirt, and odors.

7. It is comparatively economical to operate, costing less than an oil steam burner and slightly less than a coal steam boiler.

8. Utensils can be dried without removing them from the steam chest.

9. It is possible to combine chemical cleaners with the steam in one operation.

10. Hot water for washing can be provided by injecting steam, produced by this generator, into the water line.

This apparatus has the following disadvantages:

1. It does not provide for heating the milk house during the winter months except by the use of radiators.

2. It has a considerable number of moving parts to wear and to be greased.

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SOME PROPERTIES OF MILK POWDERS WITH PARTICULAR REFERENCE TO SWEET BUTTERMILK POWDERS

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Dried milk is a product of modern invention. The production of skim milk powder has grown from nothing in 1900 to over 300,000,000 pounds in 1937, while the production of dried buttermilk was about one-third of a million pounds in 1916 and over 50,000,000 in 1937. The large production and consumption of milk powders has given rise to a large number of problems relative to storage, keeping qualities, flavor control and the like.

REVIEW OF LITERATURE

Factors Affecting the Keeping Qualities of Milk Powder

The keeping quality of a food product has a direct bearing on its market value. Considerable difficulty has been experienced in keeping dried milk in good condition until it is consumed.

Palmer and Dahle (1) have suggested that the presence of air within the granules of spray process powders influences materially oxidative deterioration of whole milk powders made by this process.

Dahle and Palmer (2) later report powders exposed to moist air exhibit poor keeping qualities and give oxidation of the fat as the cause of deterioration. Time, temperature and type of container were found to influence keeping quality.

Holm, Greenbank and Deysher (3) found that precondensation and homogenization of milk improves the keeping quality of the resultant powder and conclude that the improvement is probably due to removal of the volatile oxidative catalysts by heat in the treatment. They also report progessively poorer keeping qualities with increased fat content.

Holm, Greenbank and Deysher (4) later report results indicating that clarification of milk preferably as soon as possible after drawing and high pasteurization temperatures, but not excessive ones, are conducive to good keeping qualities in the resultant powder.

Holm, Wright and Greenbank (5) report that keeping quality is largely dependent upon the inherent quality of the fat of milk powders. Their results also indicate that free moisture has a decidedly retarding effect upon susceptibility to oxidation. It was found that clarification improves the inherent quality of the fat.

Dahle and Palmer (6) find that factors known to favor oxidation proved detrimental to peroxidase activity.

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Palmer, Dahle and Macy (7) conclude that oxidative and hydrolytic decomposition and destruction of the peroxidase are accelerated by increasing the moisture content of the powders.

Supplee (8) states that staleness, decreased solubility and discoloring are more likely to occur in skimmilk than in whole milk powder, and attributes it to excessive moisture content. He found that with increased fat content there was improvement in keeping quality. This is contradictory to the results of Holm, Greenbank and Deysher.

Holm and Greenbank (9) found that the presence of water under certain conditions will retard oxidation and aids in preventing the formation of a tallowy odor. Acidity it was concluded plays a greater part in oxidation than any other one factor. Processes which remove acid therefore improve keeping qualities.

Supplee and Bellis (10) state that the development of insolubility of proteins in milk powder is due to the presence of moisture. Moisture free air was found to have no effect on solubility. Insolubility increased with increase of moisture content of powders.

Supplee (11) reports that with slow circulation of air with a relative humidity of 75 to 80 per cent through partially skimmed milk powder rapidly increased the moisture content of the powder and decreased its solubility. At moisture levels below 3 to 3½ per cent the protein of milk powder was found to remain soluble after one year.

EXPERIMENTAL

Twenty samples of various kinds of milk powders were obtained through courtesy of the Milk Producers Association of Modesto, California, and the Land O'Lakes Creamery Association. Acidity, moisture, solubility and fat determinations were made on each of these samples and an accurate record was kept of the method of manufacture and the kind of each powder. Varying percentages of sulphuric acid solutions were used to obtain a range of vapor pressure corresponding to relative humidities of 40, 60, 80, and 100 per cent. Samples representing one gram dry weight of each of the different types of powder were then placed in humidors having the above mentioned relative humidities.

Weights were taken, in most instances, daily for five days with a chemical balance, and the rate of moisture absorption determined. After the first five days weights were taken only every five days until thirty days had elapsed.

After samples were thought to have reached moisture equilibrium, those in humidors having 40 per cent relative humidities were placed in humidities of 80 per cent and then the humidity was again lowered to 40 per cent. In the case of those placed in 60 per cent relative humidity, the relative humidity was raised to 80 per cent and then lowered to 60 per cent. Dehydration studies were made on these samples. To study the effect of heat treatment on hygroscopic properties, four samples were dried by holding them in a 25-inch vacuum at 100 degrees C. for four hours. Four other samples were dried by holding them in vacuum oven for four hours at 65 degrees C. These samples were then given the same treatment as the undried samples.

The "Marquardt Method" was used in all the solubility determinations. Solubility determinations were made on samples which had been stored in various types of containers in a refrigerator and in a store room. Solubility of the fresh samples was also determined. A detailed description of the temperature of storage and the types of containers is given later. These samples were also examined for keeping qualities.

RESULTS

Hygroscopic Properties

There was a tendency for samples to reach moisture equilibrium after about 20 days. In 40 per cent relative humidity the average moisture content of all samples was 7.15 per cent, at 60 per cent relative humidity after 20 days 11.30 per cent, and after 20 days at 80 per cent relative humidity the average moisture content of all samples was 19.15 per cent. Drum dryer powders had more original moisture than did spray process powders but absorbed only about one-half as much moisture as did spray process powders at 40 per cent relative humidity. The moisture content of drum dryer powders at equilibrium was usually a little higher than that of spray process powders, however. This greater absorption of moisture by spray powders may have been due in part to the average lower fat content of the samples manufactured by the spray process. Powder made from sour buttermilk or neutralized buttermilk absorbed more moisture than sweet buttermilk powder. Skimmilk powder absorbed less moisture than either sweet or sour buttermilk powders.

A study of the effect of heat treatment of samples on their hygroscopic properties showed that dehydrated samples in general have a lower moisture equilibria at all vapor pressures tried than do undehydrated samples. Samples which were dehydrated by holding them in vacuo at 65 degrees C. reached a slightly higher moisture equilibria at 40 per cent relative humidity than did samples dehydrated by holding in vacuo at 100 degrees C. for 4 hours. The average moisture content after 40 days in a 40 per cent relative humidity of samples dried in vacuo at 65 degrees C. was 7.43 per cent, for samples held in vacuo at 100 degrees C., 6.74 per cent. At 60 per cent and 80 per cent relative humidity, the reverse was true. The samples dried in vacuo at 100 degrees C. absorbing slightly more moisture than did the samples dried at 65 degrees C. The process of heating the sample in dehydrating is thought to be the important factor in lowering its water absorbing powers and probably also accounts for the smaller amount of water absorbed by Drum dryer powders than by spray powders.

A study of the hydration-dehydration rates of both dehydrated and undehydrated samples showed the same tendencies for both types of samples. When samples were dehydrated from 40 per cent to 80 per cent relative humidity and then dehydrated back to 40 per cent, they tended to retain more moisture than they had previous to hydration. The average difference for 8 samples was 1.22 per cent. In hydrating from 60 per cent to 80 per cent relative humidity and then dehydrating back to 60 per cent, the reverse of the above situation was true. After dehydrating back to 60 per cent relative humidity the eight samples had a moisture retention average of .165 per cent less than they had previous to hydration-dehydration. The difference, however, is insignificant.

Hydration curves were much more irregular than were dehydration curves. There was a tendency for all samples to absorb moisture rapidly for the first four or five days of hydration after which time the samples lost a small amount of moisture over a period of 15 to 20 days at which time equilibrium was reached. In dehydrating, the moisture was dissipated very rapidly for the first 24 hours after which time the loss was very gradual for about 20 days at which time moisture equilibrium was reached.

A comparison of the heat treated and un-heat treated samples showed that the equilibria points for the maximum water retention by the two kinds

TABLE 1

Moisture absorbed by various samples at the several relative humidities

Sample No.	Kind of powder	Moisture content when equilibria was reached			Acidity	Fat	Method of manufacturing
		40 per cent	60 per cent	80 per cent			
		%	%	%	%	%	
1	Sweet buttermilk	7.15	11.35	19.75	0.18	8.4	Drum dryer
2	"	7.00	11.05	19.75	0.18	8.2	" "
3	"	7.00	10.90	19.70	0.18	8.4	" "
4	"	7.15	9.40	19.55	0.18	8.2	" "
5	"	7.80	11.60	22.05	0.19	5.85	Spray
6	"	8.65	10.15	19.20	0.18	5.75	"
7	"	8.10	11.95	18.25	0.15	5.25	"
8	"	7.41	10.06	18.30		5.75	"
9	"	7.87	9.93	18.66		5.60	"
10	"	6.45	9.26	21.00	0.12	5.52	"
11	"	6.55	9.25	20.42	0.13	4.80	"
12	Sour buttermilk	6.65	8.00	23.10	0.65	7.05	"
13	"	5.15	6.50	27.00	0.65	7.05	"
14	"	7.32	11.83	23.88	0.80	6.99	"
15	"	7.07	11.52	23.60	0.70	5.95	"
16	"	6.79	8.68	22.88	0.47	5.39	"
17	"	6.12	8.65	21.90	0.47	4.96	"
18	Skimmilk	7.00	8.20	17.65	0.18	0.95	"
19	"	7.20	8.30	17.65	0.18	1.15	"
20	"	7.35	10.60	17.85	0.18	1.15	Drum dryer

of samples are very near the same. However, when these samples were placed in lower humidities, the samples which had been previously heat treated or dehydrated, lost more water than those samples which had not been so treated.

The per cent of moisture taken up and retained by milk powders varies inversely as the per cent of fat. This was observed to be especially true in higher humidities.

Since fat materially affects the moisture equilibria of milk powders, a study was made of the moisture absorbed on the basis of the fat free solids of various kinds of powders.

As was to be expected, the fat free solids of drum dryer process powders were shown to absorb less moisture than did the fat free solids of spray powders when the powders tested had not been dehydrated in vacuo. When samples which had been heat treated were tested the reverse of the above situation occurred. The fat free solids of the dried spray powders absorbed less moisture than did the fat free solids of the drum dryer powders. This would indicate that the heat treatment affects the moisture absorbing properties of the spray process powders more than is the case with the drum dryer powders.

A summary of the effect of various properties of milk powders on their moisture absorbing ability is shown in Table 1.

Solubility

Table 2 shows the effect of storage on the solubility of five different milk powders, 1 A, 1 B, 1 C, 1 D, and 1 E in different containers and at different temperatures. All these powders were over 99 per cent soluble when fresh except 1 E which had a solubility of 97.5 per cent.

It seems that neutralization of sour milk increases the solubility of the resultant powder when fresh, although it dissolves considerably slower than powder made from sweet milk.

Storing in a refrigerator, at 35 to 50 degrees F. in three different containers, had very little effect on solubility of powders 1 A, 1 B, and 1 D, except the sample of 1 D which was stored in a "Sealrite" pasteboard carton and showed a solubility of 94 per cent.

There was considerable reduction in the solubility of powders 1 C and 1 E made from neutralized milk. The samples stored in containers admitting air showed the lowest solubility.

There was a very material decrease in the solubility of all the powders stored at room temperature, the greatest reduction, however, being in the powders made from neutralized milk and stored in containers admitting air. All powders, stored in a desiccator at room temperature in "Sealrite" pasteboard containers, and in open crucibles, possessed practically the same solubility after one year of storage as the fresh powder, except sample 1 C which

TABLE 2

Moisture content and solubility of buttermilk powders when fresh and after storage for one year at room temperature, and in refrigerator at temperature 35–50 degrees F.

Sample number	Container	Per cent of moisture after one year's storage				Solubility after one year's storage			
		When fresh	Refrig-erator	Store room	Store room in desiccator	When fresh	Refrig-erator	Store room	Store room in desiccator
1 A	Carton	5.37	8.65	6.1	3.95	.998	.988	.957	.994
	Sealed bottle		7.2	6.6			.977	.734	
	Cork-stoppered bottle		7.0	6.8			.991	.730	
	Crucible								.986
1 B	Carton	7.26	9.7	6.0	4.25	.995	.992	.691	.994
	Sealed bottle		6.8	7.0			.992	.674	
	Cork-stoppered bottle		7.0	5.7			.991	.694	
	Crucible								.989
1 C	Carton	8.55	10.7	6.7	5.5	.991	.624	.594	.954
	Sealed bottle		7.2	7.6			.939	.642	
	Cork-stoppered bottle		7.6	7.6			.952	.624	
	Crucible								.937
1 D	Carton	6.24	10.5	6.4	3.5	.997	.940	.928	.996
	Sealed bottle		5.2	5.4			.981	.978	
	Cork-stoppered bottle		5.4	5.6			.989	.978	
	Crucible								.996
1 E	Carton	8.58	11.5	7.5	5.4	.975	.654	.675	.973
	Sealed bottle		5.3	5.9			.955	.952	
	Cork-stoppered bottle		6.0	6.4			.958	.951	
	Crucible								.975

showed a solubility of 95.4 and 93.7 as compared to a solubility of 99.1 for the fresh powder.

A study of Table 2 would indicate that storage temperature very materially affects the solubility of the powder. Although all the powders made from sweet milk contained a high moisture content they retained high solubility when stored in a refrigerator at 35 to 50 degrees F.

Table 3 shows the moisture content of seven different powders, the solubility of these powders at two different periods, and the solubility after exposure to an atmosphere of 40 per cent relative humidity for 90 days, and after exposure to an atmosphere of 70 per cent relative humidity for 30 days.

TABLE 3

Solubility of buttermilk powders before and after being held in atmosphere of 40 per cent and 70 per cent relative humidity

Sample No.	Moisture	Solubility		Exposed to 40 per cent relative humidity for 90 days	Exposed to 70 per cent relative humidity for 30 days
		Date 2–15–28	Date 6–18–28		
1	4.27	99.5	98.0	87.2	59.0
2	4.15	99.8	99.7	91.3	54.2
3	4.38	99.7	99.8	92.4	52.4
4	4.32	99.8	99.8	93.7	55.9
5	4.30	99.5	99.5	96.8	58.3
6*	5.61	73.0	64.2	79.6	59.5
7*	5.44	95.6	79.6	78.9	58.4

* Samples 6 and 7 were made from sour buttermilk.

All the powders showed a big reduction in solubility after being exposed to 70 per cent relative humidity and all of them except No. 6, showed a lower solubility after being exposed to 40 per cent relative humidity, than they did previous to this exposure. The solubility of No. 7 made from neutralized cream was only slightly lower after exposure to 40 per cent relative humidity.

No. 6 was made from sour unneutralized milk and showed a higher solubility after being exposed for 90 days to 40 per cent relative humidity than it did at either of the two previous tests.

This would indicate that exposing powders made from sour milk to an atmosphere of relatively low humidity increases their solubility. The result of one sample, of course, is not sufficient to warrant any conclusions.

The temperature during the time of exposure of powder to 40 per cent relative humidity ranged from 60 to 80 degrees F. while the temperature during exposure to 70 per cent relative humidity ranged from 75 to 85 degrees F.

Keeping Qualities

Tables 4 and 5 show the effect of storing in different containers and at different temperatures on color and flavor of powders 1 A, 1 B, 1 C, 1 D, and 1 E.

TABLE 4

Change in color of buttermilk powders stored for one year in different containers and at different temperatures

Sample No.	Container	Color		
		Room temperature	Refrigerator 35°–50° F.	Room temperature in desiccator
1 A	Sealrite Carton	Discolored		
	Sealed glass stoppered bottle	Discolored		
	Cork stoppered bottle	Discolored		
1 B	Sealrite Carton	Discolored	Slightly discolored	
	Sealed glass stoppered bottle	Discolored		
	Cork stoppered bottle	Discolored		
1 C*	Sealrite Carton	Highly discolored	Discolored	Discolored
	Sealed glass stoppered bottle	Highly discolored	Slightly discolored	
	Cork stoppered bottle	Highly discolored	Slightly discolored	
1 D	Sealrite Carton	Slightly discolored	Slightly discolored	
	Sealed glass stoppered bottle			
	Cork stoppered bottle			
1 E*	Sealrite Carton	Highly discolored	Discolored	
	Sealed glass stoppered bottle	Slightly discolored		
	Cork stoppered bottle	Slightly discolored		

* Samples 1 C and 1 E were made from sour buttermilk.

Practically all of the samples stored at room temperature became discolored but samples 1 C and 1 E made from neutralized buttermilk contained considerably more color than the samples made from sweet buttermilk.

Samples 1 C and 1 E stored at room temperature become very yellow in color in the different containers, namely, "Sealrite" pasteboard containers, glass stoppered bottles sealed with paraffin, and cork stoppered bottles unsealed; while those powders stored in the refrigerator at 35 to 50 degrees F. were either uncolored or very slightly discolored, with the exception of 1 C stored in a "Sealrite" container.

Only powders 1 C and 1 E showed any material discoloration after being stored in refrigerator in "Sealrite" containers at 35 to 50 degrees F. None

TABLE 5

Flavor of buttermilk powders stored for one year in different containers and at different temperatures. Flavor score 10 perfect

Sample No.	Room temperature		Refrigerator temperature 35°–50° F.		Room temperature in desiccator	
1 A	Stale, musty, slightly tallowy	5½	Slightly stale	8	Slightly musty	7
	Stale, slightly fruity	5½	Slightly stale	9		
	Slightly musty, stale, slightly rubbery	5*	Stale, musty, rubbery	6*		
1 B	Slightly stale, slightly musty	6	Very slightly stale	9	Slightly fruity	7
	Slightly tallowy, slightly stale	5½	Good	10		
	Tallowy, musty, slightly bitter	5	Musty, stale	6½		
1 C**	Acid, coarse, bitter, soapy	5	Sharp, bitter, soapy, coarse, musty	5	Acid, coarse, very bitter, musty	4
	Acid, very bitter, soapy, coarse	4½	Sour, bitter, soapy, coarse, slightly stale	5		
	Acid, bitter, soapy, coarse, musty	3½	Acid, bitter, soapy, sharp, musty	5		
1 D	Stale slightly musty, very slightly tallowy	5	Slightly old, slightly stale	7	Slightly fruity slightly musty	6½
	Tallowy, stale	5	Very slightly stale, very slightly musty	7		
	Tallowy, stale	5	Slightly musty, slightly stale	7		
1 E**	Acid, stale, bitter	5	Sour, bitter, sharp, slightly musty, slightly soapy	4	Acid, bitter, cheesy, musty	3½
	Fruity, acid, bitter cheesy, musty, slightly putrid	2½	Cheesy, rubbery, acid, bitter	2½		
	Acid, bitter, cheesy, soapy	2½	Acid, cheesy, bitter, soapy	3		

* Containers had rubber stoppers.

** Samples 1 C and 1 E were made from sour buttermilk.

of the powders stored in a desiccator at room temperature in "Sealrite" containers showed any discoloration, except 1 C.

It would seem that discoloration in milk powders is a result of high temperature, moisture, and air, and powders made from sour or neutralized milk are prone to discoloration in storage.

The scores and criticism of the powders are given in Table 5. The scoring was made on the basis of 10 for the best sample. The scores were so uniformly low that it is difficult to make any suggestions except that the samples stored in refrigerator were on an average higher than those stored at room temperature. The scores of the sweet buttermilk powders averaged higher than those of the powders made from neutralized buttermilk.

Value of Sweet Buttermilk Powder as a Substitute for Gelatin

To determine the gel value of sweet buttermilk powder in ice cream five different mixes were prepared with the following composition:

38 %	total solids
15 %	sugar
12 %	fat
10.5%	serum solids
0.5%	gelatin in mixes containing gelatin

All mixes were pasteurized at 145 degrees F. for 30 minutes and homogenized at 2000 pounds pressure. The mixes were so planned as to have one mix with sweet buttermilk powder and gelatin, one with sweet buttermilk powder without gelatin, one mix with condensed skimmilk and gelatin, one mix with condensed skimmilk without gelatin, one mix with skimmilk powder without gelatin.

After freezing and hardening, one pint of the ice cream, from each mix which had been held 24 hours in cartons at about zero F., was placed on a wire gauze over a beaker at a room temperature of 78 degrees F. and the time noted required for the ice cream to melt. The conditions and appearance of the different samples on melting were also noted.

This experiment was repeated the second time.

The results of this experiment indicated that sweet buttermilk powder did not have any value as a substitute for gelatin in ice cream making.

SUMMARY AND CONCLUSIONS

1. To possess good keeping quality milk powder must be low in moisture.
2. Moisture absorption proceeds at a rapid rate at first but it required considerable time before equilibria is reached.
3. Drum dryer process powders absorb less moisture than do powders produced by the spray process.
4. The amount of moisture absorbed by powders varies indirectly as the fat content.
5. Skim milk powder absorbs less moisture than sweet buttermilk powder.
6. In an atmosphere of 100 per cent relative humidity sweet buttermilk powder may contain as much as 50 per cent moisture while powder made from sour buttermilk may contain as much as 75 per cent.
7. Moisture equilibria levels before hydration to a higher level are lower than after dehydration from these higher levels back to the lower.
8. The heating of milk powders to high temperatures in vacuo lowers their moisture equilibria levels and also limits the effect of the phenomenon observed in No. 7 to a narrow margin.
9. Sweet buttermilk powders made by the spray process are practically all dispersible, over 99 per cent, in water at 135 degrees F.

10. The development of small amounts of acid in buttermilk lowers the dispersibility of the resultant powder.

11. Buttermilk powders stored in refrigerator at 35 to 50 degrees F. had a higher dispersibility after being stored for one year than powders stored at room temperature.

12. High storage temperatures (70 to 90 degrees F.) encourage discoloration in buttermilk powders.

13. Powders made from sour buttermilk possess very poor keeping qualities and are prone to discoloration in storage.

14. Sweet buttermilk powder made by the spray process retained its original dispersibility for three years in a desiccator containing calcium chloride.

15. Sweet buttermilk powder does not possess any gel value in ice cream.

16. Sour buttermilk powder will retain more moisture than sweeet buttermilk powder when placed in desiccator containing calcium chloride.

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CONTROLLING MILK PASTEURIZATION EFFICIENCY THROUGH THE USE OF THE PHOSPHATASE TEST*

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The application of heat to milk for the purpose of destroying all pathogenic bacteria and to reduce appreciably the number of those organisms that cause spoilage and off-flavor development has become a standard practice in the dairy industry. So much emphasis has been placed upon the need of proper pasteurization in order to safeguard the health of the consumer of dairy products that it is extremely important that health officials and plant technicians be equipped with some method for accurately determining the efficiency of pasteurization.

It might be supposed that with our present system of pasteurization, there would be little occasion for errors in the process itself. However, there are instances in which inaccurate thermometers, leaky valves, and the human element have resulted in incomplete pasteurization. The industry is confronted, therefore, with the problem of securing an accurate test that will detect such discrepancies in the milk pasteurization process.

It is not too difficult to establish a series of characteristic requirements for such a test. Within all limits of practicability the test should have the following features:

1. Sufficiently accurate to detect cases where milk has not been held the required period of time nor at the required temperature.
2. Sufficiently accurate to detect even minute contamination with raw milk.
3. Rapidly performed.
4. Simple; *i.e.*, not require an elaborate layout in the way of equipment or chemicals, nor an unusual amount of skill on the part of the operator.

Kay and Graham's (1) method for determining the efficiency of pasteurization, based on the thermo-stability of the enzyme phosphatase contained in milk comes nearer to meeting the above requirements than any test previously developed. Several modifications of this test have been developed recently, which tend to increase its sensitivity and accuracy.

The object of this study was to determine the effectiveness and accuracy of the phosphatase test when applied to milk, and also to develop an accurate method for determining color intensities.

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EXPERIMENTAL PROCEDURE

Scharer's (2) modification of the original Kay and Graham test was used to determine the phosphatase content. This test is dependent upon the amount of phenol liberated by the action of the phosphatase enzyme on an excess of di-sodium phenyl phosphate during an incubation period of one hour at 37.5° C. The amount of phenol liberated is determined colorimetrically, by comparing with known phenol standards.

Photoelectric Procedure

After attempting to determine, by use of various colorimeters, the intensity of color of the phenol solutions, it was decided to attempt to develop a method which would not be dependent upon the use of the human eye to differentiate between various color intensities. A photoelectric procedure was developed which made possible the accurate determination of the amount of phenol present.

The photoelectric cell has been used extensively in various types of industrial and commercial research because of the accuracy of results obtainable. The principle of the photoelectric cell is based upon the fact that certain metals, such as sodium and potassium, have the property of giving off electrons when they are exposed to ordinary light. A photoelectric tube may be constructed by coating a portion of the inside of an evacuated glass bulb with metallic sodium or potassium. This forms one terminal of the circuit. The other terminal is a metallic electrode placed inside the bulb near the coated surface. When light falls upon the metallic coating, electrons pass to the other terminal and a flow of current results, the strength of the current depending upon the amount of light the metallic coating receives. When no light strikes the metallic coating the current ceases.

In order to select the type of photoelectric cell to be used in this procedure, it was necessary to determine the spectrum range of the solutions to be studied. Using a spectrophotometer the spectrum range of a series of phenol standards was determined. This range was found to be between 5000 and 7000 Ångstrom Units. The photoelectric cell selected as a result of this test was a Visitron F_2 showing great sensitivity in this range. Unlike various other forms of photoelectric cells, the Visitron F_2 operates without an external voltage and is intended primarily to be used without vacuum tube amplification. When light impinges upon the metallic surface, an electric current is set up producing its own E.M.F. at the terminals of the cell which causes the current to flow through an external circuit.

To further sensitize the system a glass filter with a sharp cut-off at 7000 Å was placed in front of the photoelectric cell. This glass filter allows only light of the wave length between 5000 and 7000 Å to pass on the surface of the cell.

In order to eliminate fluctuations of voltage from the line to the light source, a voltage regulator was connected between these two points. This eliminated any possible experimental error due to variations in the intensity of light passing through the colored solutions being measured in the course of the experiment.

The electrical hookup for a photoelectric cell is shown in Figure 1.

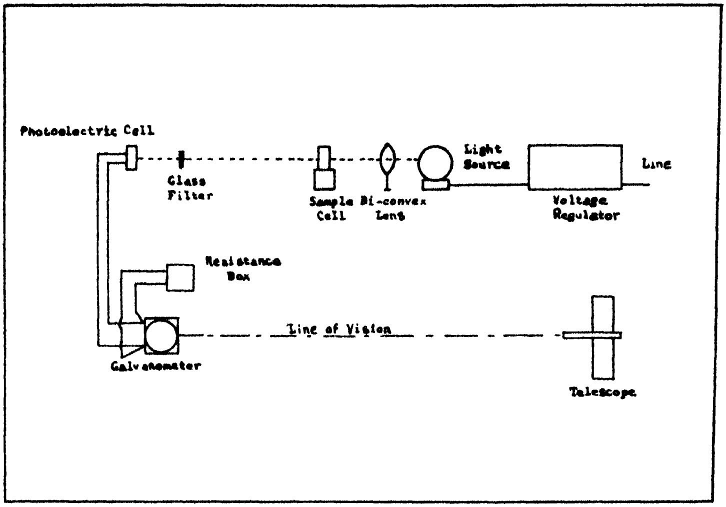

FIG. 1. Photoelectric unit.

Apparatus

1. Photoelectric Cell—Visitron Type F_2.
   G. M. Laboratories, Inc., Chicago, Illinois.
2. Glass Filter—Square Polished—No. 348. Y–4, 3⅛ in.
   Corning Glass Works, Corning, New York.
3. Glass Sample Cell—consisting of two parallel-planed Pyrex glass windows with a capacity of approximately 10 ml.
4. A 100-Watt, 115-Volt Lamp.
5. Voltage Regulator-Type VR3.
   Ritter Sales Co., Chicago, Illinois.
6. Galvanometer—Leeds and Northrup—Equipped with a special sensitive suspension.
7. Resistance Box—Leeds and Northrup Adjustable—10,000 ohms.

All apparatus was mounted on a stationary table on a floor free from vibrations.

Method of Measuring Intensities of Colored Solutions

In using the adjustable resistance box it was possible to obtain any desired deflection on the galvanometer. Due to this fact it was necessary to establish one or more standard deflections of the galvanometer which could be used every time a test was run. The method of obtaining these standard deflections was as follows:

The glass sample cell was thoroughly cleaned and rinsed with distilled water. The outer parts were dried with a soft cloth and the cell filled with 6 ml. of double-distilled water. This water was made up in a 500 ml. portion and stored at 50° F. The sample cell was placed in the holder provided for in the set-up of the equipment. The zero point on the galvanometer was adjusted. The shade was then removed from the light source which allowed the light to pass through the sample cell. The deflection of the galvanometer was adjusted using the resistance box. The water solution was removed from the cell and in its place was added a standard phenol solution which had been stored one hour at 50° F. The deflection of this solution was then determined and the per cent deflection calculated according to the following formula:

$$\frac{\text{Deflection of water solution—Deflection of unknown solution}}{\text{Deflection of water solution}} = \begin{array}{l}\text{Per cent}\\ \text{Deflection}\\ \text{of unknown}\\ \text{solution}\end{array}$$

Determining Per Cent Deflection of the Standard Phenol Solutions

Standard phenol solutions (0.5 to 10 ppm.) were prepared and the color intensity of these solutions determined using the photoelectric cell. Using water deflections of 25, 30, and 35 cm., the per cent deflections of the phenol standard solutions were calculated. All solutions were prepared in triplicate. The results are given in Table 1.

TABLE 1

Color intensity of standard phenol solutions in per cent deflection

Phenol in ppm.		Percent deflection	
	25 cm.*	30 cm.*	35 cm.*
0.50	24.20%	22.60%	22.50%
1.00	38.00	35.22	35.27
1.50	48.10	46.68	45.37
2.00	58.80	58.07	58.07
3.00	69.60	68.08	67.50
4.00	78.10	77.40	77.36
5.00	80.40	81.20	82.60
10.00	85.80	85.90	86.31

* Deflection of water solution.

As a result of this procedure a value of 35.00 cm. was selected as the standard deflection of the water solution. This value was set as the maximum and if the equipment could not be adjusted to obtain this value, then a value below 35 and above 30 was used, since the percentage deflection of the phenol standard solutions was approximately the same at these two values. Throughout the experiments conducted it was found that the deflection of the water solution could be adjusted at all times within one cm. of the value 35 cm.

The values of percentage deflection obtained using a water deflection of 35 cm. were plotted as shown in Figure 2. These values were placed on the ordinate and the corresponding phenol values on the abscissa.

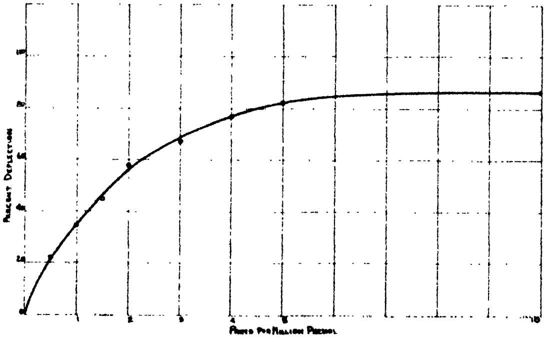

FIG. 2. Photo-cell calibration curve of phenol standards.

TABLE 2

Conversion table for phenol values

Deflection percentage	ppm. Phenol	ppm. Phenol per 1% deflection	Deflection percentage	ppm. Phenol	ppm. Phenol per 1% deflection
5.00	0.100		47.50	1.500	
		0.0200			0.0572
10.00	.200		50.00	1.643	
		0.0220			0.0868
15.00	.310		52.50	1.860	
		0.0260			0.0232
17.50	.375		55.00	1.928	
		0.0248			0.0720
20.00	.437		57.50	2.108	
		0.0252			0.0620
22.50	.500		60.00	2.263	
		0.0280			0.0624
25.00	.570		62.50	2.419	
		0.0372			0.0900
27.50	.663		65.00	2.654	
		0.0372			0.0790
30.00	.756		67.50	2.852	
		0.0448			0.0992
32.50	.868		70.00	3.100	
		0.0348			0.0950
35.00	.955		72.50	3.348	
		0.0428			0.1240
37.50	1.062		75.00	3.658	
		0.0400			0.1368
40.00	1.160		77.50	4.000	
		0.0420			0.1360
42.50	1.265		80.00	4.340	
		0.0400			0.1984
45.00	1.364		82.50	4.836	
		0.0544			0.3698

Using Figure 2, Table 2 was constructed showing the percentage deflection in one column, the corresponding parts per million of phenol in the other, and in the third column the value in parts per million of phenol for each per cent in deflection between two consecutive deflection values. Using Table 2 it is possible to calculate the color intensities of any phenol solution if the percentage deflection is known.

APPLICATION OF THE PHOSPHATASE TEST TO MILK

A study was made to determine the accuracy of the phosphatase test when applied to milk. The milk used in the following experiments was collected from a mixed herd.

Determining the Effect of Heat on the Phosphatase Content of Milk

In this experiment four lots of milk were heated to the following temperatures and held for 30 minutes: 142, 143, 144, and 145° F.

Samples were taken at the end of 15, 20, 25, and 30 minutes and immediately cooled in an ice water bath to prevent further enzyme inactivation. The phenol values obtained are given in Table 3.

TABLE 3

*Time and temperature of pasteurization as related to phenol value**

Holding period	Temperature of pasteurization			
	142° F.	143° F.	144° F.	145° F.
	Parts per million phenol			
15 minutes	3.05	2.40	1.65	0.88
20 "	1.90	1.47	0.80	0.70
25 "	1.36	1.06	0.75	0.62
30 "	1.04	0.80	0.60	0.50

* Phenol value of raw milk—5.75 p.p.m.

These data are of particular interest from the standpoint of the relative effect of the different pasteurizing temperatures at different time intervals. It is apparent from the data that variations of one degree Fahrenheit or five minutes in the holding period are detectable; however such discrepancies would be more difficult to detect at 145° F. than at 142° F. Values in parts per million of phenol obtained at the end of 30 minutes may be established as the pasteurization value of milk at each of the above temperatures; any value in excess of the above would be indicative of incomplete pasteurization. Slight discrepancies, however, could not be conclusively detected without access to the original milk in the raw state which could be pasteurized and used as a reference sample.

Effect of Contamination of Pasteurized Milk with Raw Milk

A series of experiments were performed to determine to what extent the presence of raw milk in pasteurized milk could be detected. The pasteurized samples were heated to 144° F. for 30 minutes. Typical results are given in Table 4.

TABLE 4

Detection of contamination of pasteurized milk with raw milk

Percentage raw milk	Parts per million phenol
100.00	8.80
1.00	1.80
0.50	1.20
0.40	1.08
0.30	0.94
0.20	0.81
0.10	0.69
0.00	0.60

It is evident that the addition of as much as 0.1 per cent raw milk to a pasteurized sample can be detected. It would, however, be difficult to detect this without the use of a photoelectric cell, as 0.09 parts per million of phenol difference cannot be detected by the naked eye. Addition of 0.2 or 0.3 per cent, however, may be detected without the use of the photoelectric cell.

Effect of Homogenization on the Phosphatase Enzyme

Ten gallon samples of raw milk were homogenized at two different temperatures and at various pressures. The milk was immediately homogenized after being heated, and the homogenized samples obtained immediately cooled in an ice water bath. Phenol values are given in Table 5.

TABLE 5

Effect of homogenization on the phenol value

Pressure	Temperature of homogenization	
	90° F.	143° F.
	Parts per million phenol	
No pressure	2.85	2.95
1000 pounds	2.88	2.95
1500 "	2.86	2.93
2000 "	2.86	2.77
2500 "	2.84	2.46

It is evident from these data that homogenization by pressure has no effect on the phosphatase enzyme. Although there is a small decrease in the phenol value of the sample homogenized at 143° F., this may be attributed to the fact that there was a lapse of approximately 5 minutes between the 1000 pound pressure sample and that of 2500 pounds. This 5 minute period corresponds to a 5 minute period during pasteurization and a decrease in the phosphatase activity would be expected. Several degrees rise in temperature during the homogenization process might be another contributing factor to this decrease in phosphatase activity.

Effect of Preservatives

There would be a distinct advantage in adding preservatives to the milk samples which are to be examined for the efficiency of pasteurization. In this way field samples could be sent to a control laboratory for testing, without danger of spoiling. To check the effect of preservatives on the accuracy of the phosphatase test, samples were prepared as follows:

1. Mercuric chloride—1 tablet to ½ pint of milk.
2. Sodium salicylate—1 gram to ½ pint of milk.
3. Borax—2 grams to ½ pint of milk.
4. Hydrogen peroxide—2 cc. to ½ pint of milk.
5. Formalin—2 cc. to ½ pint of milk.

The samples were run in duplicate and held at room temperature (70° to 80° F.) for a period of three days. At the end of this time it was noticed that the fat had gathered in a semi-solid mass at the top of the bottle. This condition was least noticeable in the bottle containing hydrogen peroxide. The bottles were shaken vigorously and a sample taken from each for the phosphatase test. The bottles were then placed in a hot water bath and heated to 100° F. to melt the fat, after which additional samples were taken. The average of triplicate tests on duplicate samples is given in Table 6.

TABLE 6

Relation of the presence of preservatives to the accuracy of the phosphatase test

Preservative added	Before heating	After heating to 100° F.
	Parts per million phenol	
None	0.66	0.66
Mercuric chloride	0.67	0.67
Sodium salicylate	0.92	0.91
Borax	0.70	0.70
Hydrogen peroxide	0.87	0.86
Formalin	0.60	0.59

Another test was run to determine whether the temperature of storage of the preserved samples would affect the phosphatase test. One set of the following samples was stored at a temperature of (70 to 80° F.) and another set of the same samples was stored at a temperature of (45 to 50° F.), for a period of three days.

1. Mercuric chloride—1 tablet to ½ pint of milk.
2. Sodium salicylate—2 grams to ½ pint of milk.
3. Borax—2 grams to ½ pint of milk.
4. Hydrogen peroxide—2 cc. to ½ pint of milk.
5. Formalin—2 cc. to ½ pint of milk.

The average of triplicate tests on duplicate samples is given in Table 7.

TABLE 7

Relation of storage temperature of preserved samples to the accuracy of the phosphatase test

Preservative added	Stored at 70 to 80° F.	Stored at 45 to 50° F.
	Parts per million phenol	
None		0.70
Mercuric chloride	0.70	0.69
Sodium salicylate	1.20	1.16
Borax	0.73	0.71
Hydrogen peroxide	0.83	0.81
Formalin	0.66	0.61

The results of these two experiments indicate, that of the preservatives studied, only mercuric chloride could be used as a preservative of milk without affecting the accuracy of the phosphatase test, as it consistently gave the same phenol value as that of the control sample.

Heating the preserved samples to 100° F. for the purpose of remixing the separated fat does not alter the value of the test.

Storing the preserved samples at temperatures of (45 to 50° F.) or (70 to 80° F.) had no appreciable effect on the results obtained. Although there was a consistent decrease in the phenol value of the samples stored at the lower temperatures, this variation ranges from 0.01 to 0.05 parts per million phenol and is negligible.

SUMMARY

1. Variations of one degree Fahrenheit or 5 minutes in the holding period were detected using the phosphatase test.
2. The addition of 0.10 per cent raw milk to a pasteurized sample was detected.
3. Homogenization by pressure had no effect on the phosphatase enzyme.
4. Mercuric chloride was used as a preservative of milk without affecting the accuracy of the phosphatase test. Heating the preserved sample to 100° F. to remix the separated fat had no effect on the value of the test. Storing preserved samples of milk at temperatures of (45 to 50° F.) or (70 to 80° F.) had no effect on the results of the test.

CONCLUSIONS

The results obtained in this study indicate that, within certain limits, the Scharer Test can be applied as a check on the efficiency of the pasteurization process.

No attempt was made to modify Scharer's procedure, except that a photoelectric method was developed which proved to be a great aid in obtaining definite values for the color intensities of the phenol solutions.

The need for improvement in the phosphatase test, both in method and interpretation of results is apparent, since no definite conclusions can be drawn on any sample of milk without knowledge of the temperature at which that milk has been pasteurized. There is especially a need for standardizing the method if it is to be used for condemning or accepting pasteurized milk. It is possible that, in order to make the test strictly reliable, one standard for both time and temperature of pasteurization of milk will need to be adopted.

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THE OCCURRENCE OF STREPTOCOCCUS ZYMOGENES IN THE INTESTINES OF ANIMALS

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Among the hemolytic streptococci which are encountered in the control of milk supplies are the so-called hemolytic enterococci, specifically *Streptococcus zymogenes* and *Streptococcus durans.* Because of their greater heat tolerance, these types are especially apt to be isolated from pasteurized milk in which other kinds of hemolytic streptococci are seldom found (3). The hemolytic enterococci occur rather abundantly in the intestines of normal human beings (6), and their occurrence in the intestines of other warm-blooded animals is a natural presumption. However, from the standpoint of any possible sanitary significance in milk, it is important to know definitely whether or not such hemolytic streptococci do normally occur in the intestines of animals, especially the cow.

The present work was undertaken in an effort to determine if hemolytic streptococci of the *Streptococcus zymogenes* or *Streptococcus durans* types may be present in the feces of the cow and the horse. The object was simply to answer this somewhat significant question; no effort was made to get quantitative data concerning the numbers of these organisms present, nor concerning the proportion of animals which harbor them.

Isolations were made from fresh samples of feces by two methods: One part of the sample was plated directly in poured blood agar, using several dilutions. A second portion was placed in 1 per cent sodium carbonate (7), held 24 hours at laboratory temperature, and plated in various dilutions in blood agar. Only colonies which gave broad zones of hemolysis in the blood agar were isolated for further study.

In the case of horse feces, *Streptococcus zymogenes* was isolated from one of the first three samples examined, and hence the search was carried no further. The isolation of hemolytic streptococci from cow feces proved more difficult, probably because of the occurrence in much larger numbers of the non-hemolytic *Streptococcus bovis* and *Streptococcus fecalis,* which are hardy organisms and not easily inhibited by selective procedures. However, *Streptococcus zymogenes* was obtained from one out of twenty samples of cow feces examined.

The cultures of *Streptococcus zymogenes* isolated were studied by means of all of the physiological tests which have been used for the description of this organism (4) (5) (6) and were found to be typical. They were also subjected to a serological study and found to belong to the Lancefield (1)

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group D, which group is known to include *Streptococcus zymogenes* (5) and, apparently, all of the other well-recognized types of enterococci (2).

This brief study is sufficient to show that *Streptococcus zymogenes* occurs, not infrequently, in the intestinal tracts of domestic animals. It is entirely possible that this organism is abundant in the feces of cows, but its isolation from this source is fraught with technical difficulties. Since *Streptococcus zymogenes* occurs in the bovine intestine, and since it is an extremely viable organism which can endure for long periods outside of its natural habitat, it is to be expected that it will occasionally find its way, indirectly, into milk. Its mere presence in milk in small numbers cannot, therefore, be looked upon as having a sinister sanitary significance.

SUMMARY

Streptococcus zymogenes, the most common hemolytic streptococcus in the human intestine, was isolated from the feces of the horse and the cow. The fact that this organism appears to be a normal inhabitant of the bovine intestine is of interest in connection with its occasional occurrence in milk.

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THEOPHILUS LEVI HAECKER
1846–1938

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Theophilus Levi Haecker, known as the "Father of Dairying in Minnesota," died at St. Paul, Minnesota, on August 12, 1938, at the age of 92 years. He was buried at Hampton, Iowa.

It was in the fall of 1891 that Professor Haecker at the age of 45 years came to Minnesota to begin his memorable and remarkable career as a dairy investigator and leader. His work was particularly noticeable because it was so much broader in its scope than that of a mere scientist absorbed in the intricacies of technical research. His accomplishments were so varied and his personality so human that his right to fame might rest secure on any one of his many achievements. In its effect on the dairy industry his scientific achievements may well be compared with that of the Babcock test for, like it, his discoveries in animal nutrition did away with old guess work methods. Add to this his organization work and the leadership which made Minnesota famous for its cooperative creameries and the leading butter producing state in the Union. These two, representative of his wide field of efforts, so different in character, reflect the versatile qualities of the man. The one required the studious concentration of the scientist—the other the dynamic qualities of the diplomat and salesman. In each it can be said he achieved a lasting success.

While Professor Haecker began somewhat late in life the work which was to bring him fame, yet a perspective view of his early life clearly shows that his whole previous training had really been pointed toward some such goal. He was born of German immigrant parents in a log cabin in Medina County, Ohio, on May 4, 1846. In the autumn of 1853 the Haecker family moved to a farm near Cottage Grove, Dane County, Wisconsin.

Here he attended school in a deserted lob cabin. Speaking of these early days years later, Professor Haecker said: "Most of my work was doing chores and working in the garden and helping with the housework. Mother was an expert buttermaker, and her butter was always spoken for by the year by the best customers in Madison at highest prices. She was my teacher not only in dairy manufacture but in production. Under her direction we gradually built up quite a nice dairy. Mother was superlatively orderly, neat, and industrious, and having spent so much of my younger days with her I received a training which was helpful in my personal experience in dairying."

In the autumn of 1863 he entered the University of Wisconsin, and the following March he enlisted as a drummer in the 37th Wisconsin Volunteer Infantry. After the war he went to Hampton, Iowa, where his parents had moved. Following this, he worked as a farm hand, ran a threshing outfit, attended the University of Wisconsin, taught school, published a newspaper and finally returned to Cottage Grove, Wisconsin, where he acquired what later came to be known as the Silver Springs dairy farm.

THEOPHILUS LEVI HAECKER
1846–1938

On May 11, 1874, he became a clerk in the office of Governor William R. Taylor at Madison, a position he retained for 17 years. During these years he continued to manage and develop his Silver Springs farm which soon became a veritable experiment station. He also maintained an active interest in the movement for practical agricultural education and helped to put such men as Dean W. A. Henry and Dr. Stephen M. Babcock in charge of this work at the University of Wisconsin.

At the close of Governor Hoard's administration, Mr. Haecker left the state capitol but soon thereafter enrolled at the University of Wisconsin for the first session of the dairy school—the first of its kind in the world.

Within a week he was made an instructor and put in charge of the home dairy work. Such, then, had been the training and preparation of Professor Haecker up to the time when, in June, 1891, on the recommendation of Dean Henry he was appointed by the Board of Regents of the University of Minnesota an assistant in agriculture in charge of the dairy experiments.

Professor Haecker took up his duties at University Farm, St. Paul, on October 1, 1891. From the day of his arrival until his retirement 27 years later, he devoted all his physical and mental energy to the development and advancement of dairying in Minnesota and the world. During this time he dealt with practically every phase of the industry, from the care and feeding of the herd to the manufacture and sale of the finished product. In all his research work, it was characteristic that he went to the cow herself to learn from her the secrets relating to milk production.

In the winter of 1894–95 he started the very extensive investigation which culminated in the well-known feeding standard which bears his name, the first standard of its kind to recognize that the nutrient requirement of a cow is affected not only by the quantity of milk produced but also by the percentage of fat and other solids present. This principle has since been adopted in all recognized feeding standards. The Haecker feeding standard ranks with the Babcock test in its influence on the dairy industry. This work placed Professor Haecker in the front rank of great scientists. During the last few years before his retirement, Professor Haecker devoted considerable attention to working out a similar standard for beef cattle.

During the first few years after his arrival in Minnesota, Professor Haecker spent much of his time traveling about, getting acquainted with the dairy situation in the state. One day he happened to visit a local cooperative creamery owned and operated by a colony of Danish immigrants at Clarks Grove in Freeborn County. He was greatly impressed by their success. Characteristically, he first made a careful study of the plan and organization of the creamery to determine its soundness and practicability and then took steps to vigorously promote the idea of cooperative creameries to the dairy farmers of the state.

Professor Haecker was especially apt in his ability to disseminate information whether to farmer or to student. He was in great demand as a speaker and traveled widely over the state speaking at fairs, farmers' institutes, dairy conventions and similar meetings. For many years he edited the dairy column for Farm, Stock, and Home and was a frequent contributor to Hoard's Dairyman. It has been said that he exerted an influence second to none upon the dairy industry.

He also possessed exceptional foresight and judgment and coupled with these gifts, his sincerity and common sense enabled him to assume the leadership not only among his colleagues but also of the dairy farmers of the northwest. Indeed, Minnesota's present eminence in butter production rather than in cheese is largely attributable to his vision and leadership. Recognizing the necessity of producing butter of high quality if it were to compete successfully in the markets of the country, he inaugurated short courses in buttermaking for creamery operators in which the means and methods of making high quality butter were taught. A total of about 2500 buttermakers were trained in this school by Professor Haecker.

Professor Haecker was regarded by most of his students as an inspiring teacher; to others, however, he appeared a severe taskmaster—a veritable tyrant. This, however, was only because he had no patience with indolence and slipshod methods—a heritage from his early training. Recently in speaking about this characteristic, he said, "I knew what I wanted and I had the courage to stand by until I got it. Whatever I did, I did well." To Professor Haecker the dairy cow was the most wonderful animal in the world, "God's gift to mankind." Again and again he admonished his classes, "Treat the cow kindly, boys. Remember she is a lady—and a mother." Again speaking about the cow, he said: "Surely the ways of the cow are very strange. She does and she doesn't, and then again she does neither."

Professor Haecker lived to see his early hopes and efforts come to their full fruition, a privilege accorded to but very few. During his life he was the recipient of many honors. Many dairy organizations tendered him testimonials of appreciation of his leadership and of their indebtedness to him. Undoubtedly the most prized of these was the one presented by Land O'Lakes Creameries, Inc., in June, 1935, on the occasion of the annual meeting of the American Dairy Science Association, held at University Farm, St. Paul. In 1909 he was a delegate to the international dairy convention at Budapest. The University of Wisconsin in 1923 honored him as a national leader in the fields of cooperative dairying and cattle feeding, and in 1929 he was selected by a leading dairy journal as one of the ten scientists of the world who had contributed most to the advancement of the dairy industry. In 1925, the new dairy building of the University of Minnesota was dedicated as Haecker Hall. Inside on one of its walls is a bronze plaque which bears a striking medallion portrait of Professor Haecker and with it the following tribute to him:

In Honor of
Theophilus Levi Haecker
In Charge of Teaching and
Research in Dairy Husbandry
University of Minnesota
1891–1918
For his Investigations in the
Nutrition of Dairy Cattle
And his Leadership in Organiz-
ing Co-operative Dairying
The Nation is his Debtor

American Dairy Science Association Announcements

THIRTY-FOURTH ANNUAL MEETING, PULLMAN, WASHINGTON, AND MOSCOW, IDAHO, JUNE 26–30, 1939

The Program Committee for the annual meeting is anxious to have an indication of the probable attendance. The November number of the Journal carried railroad and Pullman car rates from Chicago, Columbus, Washington and New York. If you are planning to go by rail, have you informed the Secretary of the type of transportation desired by you? Many of the members are planning to travel by automobile making the trip a vacation for the family. The Program Committee has a few touring kits left containing road maps and other travel information of interest. You are welcome to them until the supply is exhausted. By all means plan to attend this year's meeting for a glorious trip to the progressive and scenic Pacific Northwest with its Grand Coulee Dam, its beautiful lakes and streams, cool nights and hospitable people.

CALL FOR TITLES AND ABSTRACTS OF PAPERS

It has been decided to publish the abstracts of papers, to be presented at our annual meeting, in the June number of the JOURNAL OF DAIRY SCIENCE and to issue this number a little earlier than usual in order to have it in the hands of the members before leaving for the meetings at Pullman, Washington, and Moscow, Idaho. Consequently it will be necessary for us to have all abstracts in our hands by April 15. Please send titles and abstracts to Vice-Dean E. V. Ellington, Department of Dairy Husbandry, State College, of Washington, Pullman, Washington.

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THE PHYSIOLOGICAL TYPES OF BACTERIA IN MILK IN RELATION TO REDUCTION OF METHYLENE BLUE

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Since very early in the 20th century various dye reduction reactions have been used as indications of the bacterial count of milk and of its keeping quality. The reductase test has served the dairy industry admirably in providing a cheap, quick, and rather simple and accurate test, for the quality of milk. The results of the methylene blue test, however, cannot be fairly and accurately correlated with the plate count as to numbers of bacteria in the milk. This fact should not limit its usefulness.

HISTORICAL

The chemical basis of the methylene blue oxidation-reduction reaction has been the subject of a good deal of controversy. Likewise the factors involved in reaction have been disputed.

Barthel (1) believed that the disappearance of methylene blue in milk takes place in two stages, *viz.*:

1. The removal of the dissolved oxygen by the growing bacteria.
2. The reduction of the dyes by constituents of the milk. There are considerable data in support of this theory. Thornton and Hastings (7 and 8) reported it is probable that minute but inconsequential amounts of oxygen are fixed by constituents of the milk during the operation of the test. Their work confirms Barthel's theory. Therefore, the reduction time of methylene blue in raw or pasteurized milk may be looked upon as a criterion of the oxygen-consuming power of the bacteria in the milk. Thornton and Hastings (7 and 8) conclude that the reduction times of methylene blue in milk, as observed in the standard operation of their test are influenced almost entirely by the rate of oxygen uptake by the bacteria in the milk.

The rate of oxygen uptake by different species of bacteria are not well known (3, 4 and 6). Hegarty (5) reports that oxygen utilization varies greatly. Experiments reported by Bendixen and Ellington (2) showed that proteolytic, alkali-forming, inert and high lactic-acid-producing organisms reduce milk and cream as effectively as ordinary lactic acid bacteria. The

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reducing power of different organisms and of the same organisms under different conditions also appeared variable, they found.

It is probable that the rates vary sufficiently with the different species, and with different conditions, to introduce an important factor of inaccuracy into the test. This, Thornton and Hastings believe, is one of two important sources of inaccuracy, the second being the removal of bacteria from the milk by the rising butterfat.

In this paper an attempt is made to show the influence of different physiological types of bacteria on the rate of oxygen absorption and hence on the rate of reduction, and the relative number of the different varieties present at the time of reduction.

EXPERIMENTAL

Samples of milk, drawn by machine, were taken from individual cows at the University of Arizona farm. Samples were also taken of herd milk from other dairy farms. The samples were then subjected to the methylene blue test as prescribed by "Standard Methods of Milk Analysis." At the time of reduction triplicate plates were poured with standard agar in three dilutions. These plates were then incubated at 37° C. for approximately 48 hours. At this time a colony count was made using a hand lens. All plates were counted when possible and an attempt was made to correlate the counts of one plate with another. After counting, the plates were marked off in four different equal portions. The quarter portion having the most evenly distributed colonies was then selected and 25 consecutive colonies from this portion were inoculated into litmus milk, being careful that the colonies were representative of the entire plate. These inoculated litmus milk tubes were then held at laboratory temperature for periods running from 4 to 7 or 8 days, according to the temperature. The temperature of the laboratory seldom goes below 60° F. in the winter or above 90° in the summer. As soon as bacterial action made it possible to detect the physiological types of bacteria in the tubes, the tubes were examined and classified. The tubes were classified as containing acid-coagulating, acid-not-coagulating, acid-coagulating-gas-proteolytic, acid-proteolytic, alkaline, and inert bacteria and a record was also made of the blank tubes in which no action occurred. Blank tubes and tubes containing inert bacteria were determined by making agar slants of the medium.

Accurate records were kept of the date when the samples were taken, the cow or herd from which they were taken, the time required for reduction of methylene blue, the plate count after reduction, and the physiological types of bacteria present. Over 1,000 different samples in all were run, making more than 25,000 tubes that were inoculated.

On about ⅓ of these samples plate counts were made before subjecting the samples to the methylene blue test and the physiological types of bacteria were determined as above to determine which organisms were most active in growth activities in the methylene blue milk.

In all the experimental work, a concerted effort was made to keep all instruments used in a sterile condition and to prevent outside contamination of samples. Sterile 1 cc. and 10 cc. pipettes were used for drawing methylene blue and milk into the tubes. Sterile 30 cc. test tubes were used for making the test and for holding inoculations.

RESULTS

Normal Proportion of Different Types of Bacteria in Milk

In summing up the results of the 25,000 inoculations the results show an average of 6.27 per cent acid-coagulating, 55.82 per cent acid-not-coagulating, 11.59 per cent acid-coagulating-gas-proteolytic, 16.56 per cent acid-proteolytic, 2.68 per cent alkaline, 2.83 per cent inert, and 4.25 per cent blank tubes. This milk was produced under sanitary conditions but came from different farms and was produced under different conditions. The plate count of the milk before reduction varied from about 2,000 bacteria per cc. up to as high as 30,000 per cc. with an average count of about 8,000 per cc. This is thought to be fairly typical of most grade A raw milk produced under the same conditions and the bacterial flora can for all practical purposes be considered to be about the same in all such grade A milk under normal conditions. It was found that the approximate average reduction time for this milk was, in round numbers, about 6 hours, but varied from

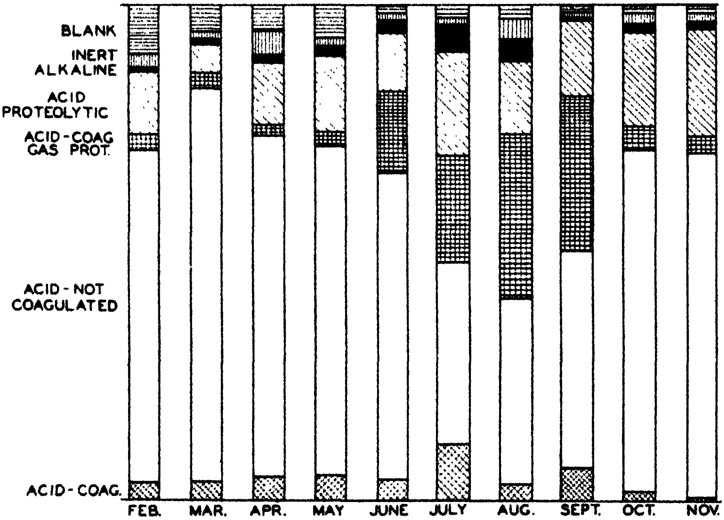

FIG. 1. This shows the wide seasonal variation in types of bacteria. Note the acid-coagulating-gas-proteolytic and acid-not-coagulating types particularly.

about 45 minutes up to as high as 14 and 15 hours in rare instances. The blank tubes are considered as having been inoculated with bacteria which find milk an unsuitable medium for growth, or it may be that the colony may have been missed by the inoculating needle in making the inoculation. Considerable difficulty was encountered with pin-point colonies, especially during the summer months, which made it hard to make certain of inoculations.

Seasonal Variations in Type

It was found (see Figure 1) that during the spring and summer months the number of acid-not-coagulating bacteria constantly decreased until fall.

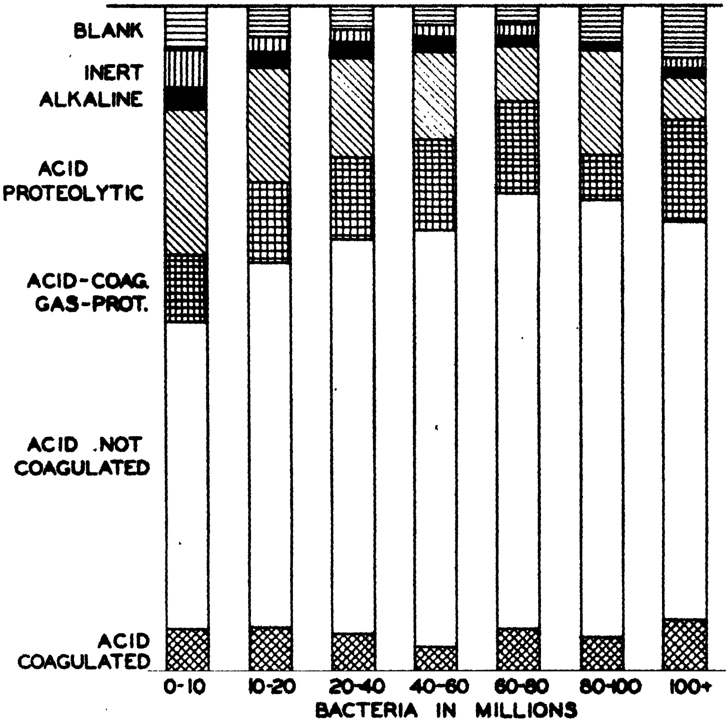

FIG. 2. This shows the physiological types of bacteria according to the bacterial count after reduction.

During this time the number of acid-coagulating bacteria, the alkaline and particularly the acid-coagulating-gas-proteolytic bacteria increased. The blank, inert, and acid-proteolytic types varied considerably from month to month but the variation was not uniform. From September until March the variation in type was exactly opposite. This is thought to be of significance in view of the fact that the average time for reduction decreased during the spring and summer and increased during the winter months, showing approximately the same trend as the acid-not-coagulating types and a trend exactly opposite to that shown by the acid-coagulating, alkaline, and acid-gas-coagulating-proteolytic types.

Relationship between Plate Count after Reduction and Type

The data showed that as the plate count after reduction increased (see Figure 2) the number of acid-not-coagulating bacteria increased, the number of acid-coagulating-gas-proteolytic bacteria increased as a rule and the number of alkaline, inert and acid-proteolytic bacteria decreased. The other types varied but not uniformly. These observations were to quite an extent in agreement with what we had expected in accordance with other observations. No conclusions can be drawn, however, from this particular phase of the study without first taking some other comparisons into consideration. It was noted that 16.87 per cent, 28.44 per cent, 35.05 per cent, 14.34 per cent, 3.23 per cent, 1.22 per cent, and .88 per cent of all the samples observed had from 0–10, 10–20, 20–40, 40–60, 60–80, 80–100 and above 100 million bacteria per cc. respectively, after reduction. The average samples had from 20 to 40 million bacteria per cc. at the time of reduction.

Relationship between Time of Reduction and Type

Bendixen and Ellington (2) observed that the reducing power of different organisms and of the same organism under different conditions appeared variable. This observation was strikingly substantiated when an attempt was made to show any relationship between time of reduction and type. The percentages of the different organisms varied greatly as the time but the variation in no instance showed any particular trend. This was contrary to expectation. This leads us to believe there is an association action between the different types of bacteria, and that the rate of oxygen absorption by the different types of bacteria is very materially influenced by their co-habitation with other types of bacteria. This belief has been substantiated by other workers.

Time-Count Relationships

A complete biometrical study of the data was made in an attempt to find a relationship between reduction time and type, and between count and type. In most instances there was no general trend for any of the types of bacteria, as the bacterial count increased. It was noted, however, that in most instances the acid-not-coagulating type of bacteria showed a tendency to increase and that the acid-proteolytic type tended to decrease as the count increased. This tendency was not constant. For other types, no such tendencies could be found. In a further study it was noted that in the shorter reduction times the acid-not-coagulating types were not prevalent and that the acid-proteolytic types and acid-coagulating types predominated. Just the opposite of this was true in longer reduction times.

On the basis of these time-count type studies of both the count in thousands before reduction and the count in millions after reduction and the physiological types both before and after reduction it was concluded that there is no very close relationship between physiological type and reduction time.

Growth Rates During Reduction Test

In order to see if any specific types were more active in their growth activities than other types, charts[1] were prepared using count classifications in millions after reduction of from 0–5 million, 5–10, 10–20, 20–30, 30–40, 40–50, 50–100, and 100 million and above to show the relationships of percentages of the various physiological types both before and after reduction. A study of this chart showed that in general the percentage of acid-coagulating, acid-proteolytic, alkaline, inert, and blank tubes decreased during the reduction process. The number of acid-not-coagulating and acid-coagulating-gas-proteolytic types increased. The tendency of the acid-coagulating-gas-proteolytic types to increase became less and less as the bacterial count increased, while the tendency on the part of the other types to either increase or decrease remained nearly constant for all bacterial count classifications.

A similar study was made classifying the bacteria both before and after reduction according to time of reduction. Reduction times of from 3 to 4 hours, 4–5, 6–7, 7–8, 8–9 hours and above were used. Increases and decreases of the various types similar to those found in the previous study were observed.

It was noted, however, that as the count went up the percentage of acid-coagulating-gas-proteolytic types increased while the percentage of acid-not-coagulating types decreased. As the time for reduction increased there was an exactly opposite trend, the acid-coagulating-gas-proteolytic types decreased while the number of acid-not-coagulating types increased. This is thought to be significant.

Table 1 shows the proportion of the various types before reduction to the

TABLE 1

Changes of percentages of types during the reduction process

Types of bacteria	Acid-coagulating	Acid-not-coagulating	Acid-coagulating-gas-proteolytic	Acid-proteolytic	Alkaline	Inert	Blank
Before reduction	7.3%	22.9%	31.3%	23.3%	5.8%	5.1%	4.2%
After reduction	1.7	41.5	37.9	12.9	1.6	2.1	2.2

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[1] A copy of these charts can be had by writing the authors.

same type after reduction of those samples in which both studies were made. Count and reduction time were not considered in making these comparisons. This table shows increases and decreases in numbers similar to those found in the other studies.

A study of the relationship between the original plate count before reduction and the count after reduction as well as the reduction time was made. It was found that there is no direct relationship between the count before and the count after reduction. There is a relationship, however, between the original count and the time of reduction. As has always been found by other workers, as a general rule the time for reduction varies inversely with the original count. The authors found numerous exceptions to this rule, however.

Table 2 shows the relationship between the time of reduction and the count after reduction. It will be noticed that, as the time for reduction increased the count also increased. This was in accordance with what we had expected on the basis of other studies. It shows clearly that one or more types present must have an inhibitory effect on one or more of the other types. So far we have been unable to show any close relationship between type and reduction time.

TABLE 2

Bacterial count after reduction according to reduction time

Reduction time	Average count after reduction
0– 2 hours	10,000,000 per cc.
2– 3 "	17,000,000 " "
3– 4 "	19,000,000 " "
4– 5 "	21,000,000 " "
5– 6 "	21,000,000 " "
6– 7 "	23,000,000 " "
7– 8 "	22,000,000 " "
8– 9 "	33,000,000 " "
9–10 "	37,000,000 " "
10–11 "	36,000,000 " "
Above 11	61,000,000 " "

Type Variations in Individual Samples

The variation in type found in individual samples was considerable. No close relationship between time of reduction and type was found. It may be concluded that differences in reduction time for different samples bear no direct or great relationship to the types of bacteria present.

DISCUSSION

It seems that there is a very marked seasonal variation in the physiological types of bacteria in milk. Part of this variation may have been due, however, to seasonal variations in the laboratory incubation temperature. The decline in reduction time during the spring and summer months would indicate that the change is one of type rather than of growth rates due to temperature changes. The temperature change in the laboratory in which the litmus milk tubes were incubated may have played a small part in the physiological type change. Nevertheless, the decrease in the number of acid-not-coagulating type of bacteria which followed the decrease in time of reduction would indicate that acid-not-coagulating types are poor reducers. On the other hand, during the same period in which the acid-not-coagulating types decreased there was an increase in the number of alkaline and acid-coagulating-gas-proteolytic types of bacteria which would indicate that they are primarily important in reducing.

Barthel (1) points out that reduction does not occur until oxygen has been partly absorbed. This would indicate that acid-not-coagulating types of bacteria are poor utilizers of oxygen and that alkaline and acid-coagulating-gas-proteolytic types are rather important in oxygen utilization.

The study shows, however, that acid-not-coagulating types of bacteria predominate in good quality raw milk and so must play a large part in reducing the dye. The acid-coagulating-gas-proteolytic types make up only 11.59 per cent of all bacteria in the samples of this study but as shown above must play a rather important rôle in reduction of methylene blue and oxygen absorption. The small percentage of alkaline bacteria (2.83 per cent) precludes their being of much importance as regards reduction time.

The study shows that as reduction time goes up, the plate count of bacteria after reduction also increases. (See Table 2.) It was also shown that as the count goes up, the acid-not-coagulating types of bacteria increase and proteolytic types of bacteria decrease. This tends to verify the above assumptions that acid-not-coagulating types are slow reducers and oxygen absorbers while proteolytic types are rather hasty oxygen absorbers. (See Figure 2.)

A study of the growth rate variations during reduction, according to bacterial count after reduction again verifies the above assumptions. The acid-not-coagulating types were seen to increase in number as reduction time increased. It is significant, however, that there was an increase in percentage of bacteria of this type after reduction over the percentage before reduction, but that as reduction times increased this tendency decreased. This indicates that if this type of bacteria was present in small amounts before reduction in relation to other types reduction time was short in proportion to the number present. As the number increased before reduction, the time for reduction was increased until at from 9–10 hours for reduction, the number before reduction and after reduction was nearly the same. The important thing, however, is that the acid-coagulating-gas-proteolytic types showed a tendency exactly opposite to that shown by the acid-not-coagulating types. This we think is very substantial evidence as to the absorption and reduction powers of the two types of bacteria.

Since the plate count of the bacteria increases after reduction as reduction time increases (see Table 2) it can be assumed that the bacteria in those samples having a high reduction time are rather inefficient in reducing methylene blue. Acid-not-coagulating types increase as the count after reduction increases.

The time-count comparisons, however, fail to show any tendency for any particular type to be especially important in reducing powers. This, we feel, shows that there is definitely an associative action between bacteria, some types having an inhibitory or possibly an accelerating effect on other types. There was a general tendency for acid-not-coagulating types to increase as time and count increased. The tendency for proteolytic types was exactly opposite. This accelerating and inhibitory effect that co-habitating bacteria have on one another is a common bacteriological phenomenon.

If various types of bacteria do have varying oxygen absorption powers as various workers have found they do (7 and 8), then variations in bacterial flora from individual cows or in herd milks could be assumed to be a source of inaccuracy, when estimating counts on the basis of this test. This study failed to show that such variations are the source of inaccuracy. The associative action among bacteria seems to overshadow any special reducing or oxygen absorptive powers of various physiological types of bacteria.

Since proteolytic types of bacteria have been shown to have some special reducing power, it seems that the ability of the methylene blue test to indicate quality of milk is strengthened. Proteolytic types of bacteria in milk are particularly objectionable especially from the standpoint of cheesemakers and producers of other by-products which must be stored for long periods of time.

SUMMARY

1. Over 1,000 milk samples were studied for physiological types of bacteria present in relation to reduction time of methylene blue.
2. The samples studied were shown to contain on the average of 6.27 per cent acid-coagulating bacteria, 55.82 per cent acid-not-coagulating, 11.59 per cent acid-coagulating-gas-proteolytic, 16.56 per cent acid-proteolytic, 2.28 per cent alkaline, 2.83 per cent inert bacteria and 4.25 per cent of our tubes were blank.
3. There is a wide seasonal variation in physiological types of bacteria in milk.
4. Acid-not-coagulating bacteria are poor reducers of methylene blue but are predominant in most high quality milk.
5. Proteolytic types of bacteria are good oxygen absorbers and play an important part in reduction.
6. There is an associative action between bacteria which usually overshadows individual variations of reduction power.

7. Marked variations in physiological types of bacteria present in individual samples failed to have any material effect on reduction time.

8. Acid-coagulating, alkaline and inert bacteria seem to have no special effect on reduction rates, unless present in unusually large numbers.

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DETERMINING THE EFFICIENCY OF ICE CREAM MIX PASTEURIZATION THROUGH THE USE OF THE PHOSPHATASE TEST

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The use of the phosphatase test for determining the efficiency of milk pasteurization has been studied by Kay and Graham (1), Gilcrease and Davis (2), Hahn and Tracy (3) and others. However, the literature contains no reference to the application of this test to ice cream. Since ice cream is a mixture made from cream, milk, condensed milk or milk powder, sugar, gelatin, coloring and flavoring, it does not necessarily follow that the results of the phosphatase test on ice cream would be comparable to those obtained on milk.

The possible combinations of ingredients used in making a mix are unlimited. It was necessary, therefore, for the purposes of this study to select a standard mix from the standpoint of both composition and ingredients used. Unless otherwise specified, the milk products used were raw cream, raw skim milk, and milk powder. All mixes contained 12 per cent fat, 10 per cent serum solids, 15 per cent sucrose and 0.3 per cent gelatin, except in those cases where the nature of the experiment was such that it was necessary to deviate from this standard. In an earlier study by the authors (3), homogenization by pressure was found to have no relation to the phosphatase test, and since it facilitated matters to do so, homogenization was omitted in the preparation of the experimental mixes. All mixes were pasteurized in the laboratory.

The Scharer Test was used to determine the phosphatase content. This test is based on the measurement of the amount of phenol liberated by the action of the phosphatase enzyme on an excess of di-sodium phenyl phosphate during an incubation period of one hour at 37.5° C. The amount of phenol liberated was determined colorimetrically by comparing with known phenol standards. A photo-electric procedure, explained in detail in an earlier publication of the authors (3) was used to measure color intensities of the phenol solutions. For this procedure a series of phenol standards (0.5 to 10.0 p.p.m.) were prepared. These were stored in a dark room at 50° F. for a period of one hour before being used. A glass sample cell was filled with double distilled water and placed in the photoelectric unit and the amount of deflection obtained on a galvanometer. The sample cell was then emptied of the water and filled, in turn, with each of the standard phenol solutions, and the deflection measured. The percentage deflection was calculated according to the following formula:

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$$\text{Percentage deflection of phenol solution} = \frac{\text{Deflection of water solution} - \text{Deflection of phenol solution}}{\text{Deflection of water solution}}$$

The values obtained were plotted on a graph with their corresponding values in parts per million phenol, the deflection percentage being plotted on the ordinate and the parts per million of phenol on the abscissa. A smooth curve was obtained which represents the calibration curve of the photoelectric unit.

In determining color intensities of unknown solutions, the same procedure was followed, substituting the unknown solutions in place of the phenol standard solutions. The percentage deflection of the unknown solution was determined and the corresponding parts per million phenol interpolated by using the calibration curve.

THE RELATION OF TEMPERATURE OF PASTEURIZATION TO PHENOL VALUE

Six lots of mix were pasteurized at temperatures varying from 143° to 160° F. Samples were taken at the end of 15, 20, 25 and 30 minutes and tested in triplicate. Typical results of a series of tests are given in Table 1.

TABLE 1

Time and temperature of pasteurization as related to the phenol value

Holding period	Temperature of pasteurization					
	143° F.	145° F.	147° F.	149° F.	150° F.	160° F.
	Parts per million phenol					
Raw	3.66	3.66	3.66	3.66	3.66	3.66
15 minutes	3.50	2.65	2.26	1.09	1.10	0.48
20 minutes	3.34	2.60	1.97	0.68	0.61	0.41
25 minutes	3.23	2.47	1.64	0.47	0.40	0.40
30 minutes	3.10	2.38	1.33	0.33	0.32	0.32

These data are of particular interest from the standpoint of the relative effect of the different pasteurizing temperatures at different time intervals. Temperatures of 143° F. and 145° F. inactivated to only a small degree the amount of phosphatase present in the mix. At 147° F. there was a noticeable increase in the inactivation of the enzyme while at 149° to 160° F. the inactivation was what might be considered complete, with no appreciable differences in the value obtained at the end of the 30-minute holding period. However, slight discrepancies in the holding period would likely be more noticeable at 149° and 150° than at 160° F.

EFFECT OF USING PARTIALLY PASTEURIZED INGREDIENTS UPON PHENOL VALUE

Two different types of the standard mix were made. One mix was prepared from milk products that were in a fresh, raw state, and the other mix was made from milk products which had been heated to a temperature of 150° F. for 15 minutes. A set of each of these mixes was heated at 143°, 150° and 160° F. Samples were taken at the end of 15, 20, 25 and 30 minutes and tested in triplicate. Typical results of a series of tests are given in Table 2.

TABLE 2

Effect of using raw or partially pasteurized ingredients in the mix as related to the phenol value

Holding period	Temperature of pasteurization					
	143° F.		150° F.		160° F.	
	Raw	Part past.	Raw	Part past.	Raw	Part past.
Starting mix	3.65	0.91	3.65	0.91	3.65	0.91
15 minutes	3.48	0.90	0.95	0.66	0.51	0.36
20 minutes	3.40	0.75	0.66	0.62	0.40	0.34
25 minutes	3.21	0.65	0.43	0.41	0.32	0.32
30 minutes	3.10	0.61	0.31	0.31	0.31	0.31

It is interesting to note the extreme difference in the final phenol value of the mix made from raw milk products and that mix which contained partially pasteurized milk products, when these mixes were pasteurized at 143° F.

Apparently the presence of protective solids in the ice cream mix has a prohibitive effect on the inactivation of the phosphatase enzyme. The heat treatment to which a mix is subjected when pasteurizing at temperatures of 143° F. is not sufficient to overcome this protective effect, and therefore, incomplete inactivation of the enzyme results. Referring to Table 1, it is evident that the same condition would exist when pasteurizing mix at temperatures as high as 147° F. When pasteurizing at temperatures of 150° F. or above, the heat treatment apparently was sufficient in all cases to overcome the protective effect of the mix solids, since no variations were obtained in the final phenol value of the mix made from all raw milk products and that mix made from partially pasteurized milk products.

COMPARISON OF PHENOL VALUE OBTAINED ON MIX AND MILK SUBJECTED TO THE SAME HEAT TREATMENT

A batch of standard mix made from all raw milk products and the same volume of raw four per cent milk were pasteurized at three different temperatures. Samples were taken after 10, 20 and 30 minutes. Tests were run in triplicate, the phenol values being given in Table 3.

Subjecting milk and ice cream mix to the same heat treatment apparently had no varying effect at temperatures above 150° F., as in both cases approximately the same final phenol value was obtained. At a temperature of 143° F., it is evident that the heat treatment was not sufficient to inactivate the enzyme present in the mix to the same extent as that present in the milk due to the protective action of the additional solids present in the mix.

TABLE 3

Comparison of mix and milk as related to the phenol value

Holding period	Temperature of pasteurization					
	143° F.		150° F.		160° F.	
	Mix	Milk	Mix	Milk	Mix	Milk
	Parts per million phenol					
None (control)	3.80	4.50	3.80	4.50	3.80	4.50
10 minutes	2.95	2.11	1.26	0.68	0.46	0.37
20 minutes	2.85	1.10	1.54	0.50	0.40	0.36
30 minutes	2.65	0.73	0.32	0.35	0.32	0.32

EFFECT OF THE CONDENSING OPERATION UPON THE PHOSPHATASE CONTENT OF CONDENSED MILK

Since condensed products are widely used in making ice cream mixes, the effect of the condensing operation on the phosphatase content of milk would be of interest. In order to determine this effect, the phenol value on several batches of condensed milk was determined. These products were pasteurized at temperatures ranging between 143° and 150° F. for 30 minutes before entering the vacuum pan. The condensing process was carried on at temperatures between 140° and 145° F., and the time in the pan was not less than 40 minutes. The lowest value obtained on a finished product was 0.25 parts per million phenol and the highest value, 0.37 parts per million. In Table 4 are given the results of the two high and low batches. Composition of condensed skim milk was 32 per cent total solids and condensed whole milk, 8 per cent fat, 20 per cent milk-solids-not-fat.

TABLE 4

Effect of the condensing process as related to the phenol value

Product	Pasteurizing temp.	State of milk product			
		Raw	After pasteurization	After condensing	Last drawn from pan
		Parts per million phenol			
Skim milk	150° F.	4.00	0.31	0.25	0.25
Whole milk	143° F.	4.22	0.65	0.37	0.37

The results obtained indicate that the condensing process appreciably reduced the phosphatase content of the milk product.

EFFECT OF THE SERUM SOLIDS CONTENT OF THE MIX ON PHENOL VALUE

Three batches of mix were prepared, using as milk products fresh raw cream 30 to 40 per cent fat, fresh raw skim milk, and skim milk powder. The mixes were made so as to have the following composition:

1. 12 per cent fat, 9 per cent serum solids, 15 per cent sugar, 0.30 per cent gelatin.

2. 12 per cent fat, 12 per cent serum solids, 15 per cent sugar, 0.30 per cent gelatin.

3. 12 per cent fat, 15 per cent serum solids, 15 per cent sugar, 0.30 per cent gelatin.

All mixes were heated to 150° F. and samples were taken after 15, 20, 25 and 30 minutes. Samples were tested in triplicate, the results being given in Table 5.

TABLE 5

Effect of the amount of serum solids in the mix as related to the phenol value

Holding period	Per cent serum solids		
	9%	12%	15%
	Parts per million phenol		
15 minutes	0.40	0.43	0.47
20 minutes	0.36	0.39	0.39
25 minutes	0.34	0.35	0.36
30 minutes	0.31	0.31	0.32

When pasteurized at 150° F. for 30 minutes the percentage of serum solids in the ice cream mix had no effect on the final phenol value. It was found advisable, when testing mix of high serum solids content, 12 per cent and above, to use 0.15 cc. instead of the usual 0.10 cc. of lead acetate to completely precipitate the protein.

EFFECT OF STORING MIX ON PHENOL VALUE

Two batches of pasteurized, unflavored and uncolored ice cream mix, one 12 per cent fat, and the other 16 per cent fat, were stored at 40° F. for a period of two weeks, and a phosphatase test made on each batch periodically. All samples were run in triplicate. Phenol values are given in Table 6.

TABLE 6

Effect of storing ice cream mix at 40° F. as related to the phenol value

Mix	Start	Period of time in days								
		1	2	3	4	5	7	11	13	15
	Parts per million phenol									
12% fat	0.38	.38	.37	.37	.37	.36	.34	.34	.35	.35
16% fat	0.34	.34	.34	.34	.35	.33	.31	.31	.32	.32

Storing ice cream mix at 40° F. for a period of two weeks did not appreciably alter the phenol value. In case of the 12 per cent mix a reduction in phenol value of 0.03 parts per million occurred, while the 16 per cent mix reduced 0.02 parts per million. Such small reductions are within range of experimental error and, therefore, are irrelevant in this case.

EFFECT OF FREEZING UPON PHENOL VALUE OF MIX

A batch of mix was pasteurized at 150° F. and sample portions taken at the time the temperature reached 150° F. and again after holding for 15, 20, 25 and 30 minutes. The unflavored and uncolored mixes were aged for two days at 40° F., frozen in a hand freezer and stored at −20° F. The ice cream was sampled after the first and fourth days, and the first, third, eighth and twelfth week of storage. Before testing, the samples of ice cream were heated in a water bath to 100° F. and thoroughly mixed so that as much air as possible could be eliminated. Tests were run in triplicate. The data are given in Table 7.

TABLE 7

Effect of storing mix and ice cream as related to the phenol value

Period of test	Raw	Period of pasteurization at 150° F.				
		0 min.	15 min.	20 min.	25 min.	30 min.
	Parts per million phenol					
Fresh mix	3.66	3.17	0.90	0.62	0.43	0.31
Mix aged 2 days at 40° F.	3.40	2.95	0.88	0.62	0.44	0.35
Ice cream from freezer	3.40	2.95	0.91	0.62	0.43	0.315
At −20° F. for 1 day	3.10	2.65	0.90	0.61	0.43	0.31
" " " 4 days	3.10	2.65	0.88	0.61	0.44	0.32
" " " 1 week	3.10	2.65	0.82	0.62	0.44	0.32
" " " 3 weeks	2.80	2.55	0.88	0.60	0.43	0.34
" " " 8 weeks	2.65	2.41	0.80	0.58	0.41	0.31
" " " 12 weeks	2.63	2.40	0.75	0.56	0.42	0.31

Storing raw mix at 40° F. for two days decreased the phenol value. The same was true for the sample heated to 150° F. but not held. Samples of mix taken at the end of 15 minutes or longer, during the holding period, showed no appreciable change in the phenol value after two days' storage at 40° F.

Storing ice cream at −20° F. over a period of 12 weeks resulted in marked decreases in the phenol values of all the samples except those taken after 20, 25 and 30 minutes in the holding period. The underpasteurized samples after 12 weeks' storage still gave a strong test for phosphatase.

EFFECT OF FLAVORING MATERIALS ON THE ACCURACY OF THE PHOSPHATASE TEST

Five different types of vanilla flavoring materials were added to pasteurized ice cream mix and these samples stored at 40° F. After a period of 1, 3, 7 and 14 days, a phosphatase test was run on the samples. The results of this experiment are shown in Table 8.

Pure vanilla had no effect on the phenol value while the imitation and artificial compounds increased the phenol value to a small degree. Coumarin, however, increased the phenol value approximately 15 times and vanillin showed an increase of double the control sample.

TABLE 8

Effect of vanilla flavoring on the phenol value of ice cream mix

Vanilla	Storage period at 40° F.			
	1 day	3 days	1 week	2 weeks
	Parts per million phenol			
Control	0.31	0.31	0.31	0.31
Pure	0.31	0.30	0.30	0.30
Imitation compound	0.38	0.36	0.36	0.36
Pure and vanillin	0.36	0.35	0.35	0.355
Vanillin	0.60	0.53	0.53	0.52
Coumarin	4.50	4.00	3.60	2.81

In further experiments six different flavoring extracts were added to pasteurized mix and the samples were stored at 40° F. Tests were made on these samples after one, three and seven days in storage. The results are shown in Table 9.

TABLE 9

Effect of the addition of flavor extracts to ice cream mix as related to the phenol value

Extract	Storage period at 40° F.		
	1 day	3 days	1 week
	Parts per million phenol		
Control	0.31	0.31	0.31
Pineapple	0.315	0.315	0.31
Orange	0.325	0.32	0.32
Cherry	0.32	0.32	0.32
Lime	0.32	0.32	0.315
Strawberry	0.34	0.34	0.33
Maple	0.32	0.32	0.35

In five out of six cases the phenol value was increased with the addition of flavor extracts; however, the increase was too small to be considered significant.

EFFECT OF "COLD-PAK" FRUITS UPON PHENOL VALUE

Three different "Cold-Pak" fruits were added to ice cream mix which was then stored at 40° F. Tests were made after one, three and seven days' storage. The flavored samples all soured after one week. Results of this study are shown in Table 10.

TABLE 10

Effect of the addition of "cold-pak" fruits to ice cream mix as related to the phenol value

Fruit	Storage period at 40° F.		
	1 day	3 days	1 week
	Parts per million phenol		
Control	0.30	0.30	0.30
Cherries	0.40	0.42	0.50
Strawberries	0.41	0.41	0.44
Pineapple	0.40	0.41	0.47

It is interesting to note the increase in phenol value in the fruit ice creams during the storage period. This increase may have been caused by bacterial action on the ice cream, resulting in the liberation of phenolic compounds.

EFFECT OF THE ADDITION OF COLOR UPON THE PHENOL VALUE

Numerous experiments were performed to determine the effect of the addition of color to the ice cream mix upon the phenol value. The results obtained varied to such an extent that no definite conclusions could be made.

The coloring material itself had a tendency to be precipitated with the protein on the addition of lead acetate to the substrate-ice cream mixture. If the entire amount of coloring material present was removed in this manner, the phenol value obtained was the same as that of the control sample. However, the precipitation of the color with the protein material by lead acetate cannot be controlled by the operator and in some cases, some of the coloring material passes into the clear filtrate with the resultant increase in the color intensity. Although in several cases the addition of coloring to ice cream mix had no effect on the phenol value, this was not always true. The fact cannot be overlooked that there exists a possibility of incomplete removal of the coloring material with the lead acetate-protein precipitate, so that the use of the phosphatase test to determine pasteurization efficiencies of ice cream to which a coloring material has been added may produce erroneous results.

APPLICATION TO COMMERCIAL ICE CREAM SAMPLES

In order to check the efficiency of pasteurization of ice cream being sold commercially, samples of unflavored ice cream mix were collected from eight different ice cream distributors. A phosphatase test was run on each, the results being given in Table 11.

TABLE 11

Results of phosphatase test on commercial samples

Distributor	Phenol value in parts per million
1	0.32
2	0.31
3	0.32
4	0.32
5	0.28
6	0.35
7	0.31
8	0.29

In all cases but one (Distributor 6) the phenol values obtained were 0.32 parts per million or below, indicating complete pasteurization of the mix had taken place.

SUMMARY

1. Using the phosphatase test, as modified by Scharer, it was found possible to detect variations in the time and temperature of pasteurization of ice cream mixes, provided they had not been colored or flavored.

2. The use of all raw or partially pasteurized milk products in building a mix had no effect on the final phenol value, if the mix was pasteurized at temperatures of 150° F. or above. However, at temperatures of 147° F. or below, the mix made from all raw milk products gave a considerably higher final phenol value than in the case of the mix made from partially pasteurized milk products.

3. Subjecting raw milk and ice cream mix made from all raw milk products to the same heat treatment by heating to temperatures of 150° F. or above and holding for 30 minutes resulted in the same final phenol value for both the milk and mix. Temperatures below 150° F. resulted in a higher final phenol value for the ice cream mix than that of the milk.

4. The condensing process decreased the amount of phosphatase enzyme present.

5. Increasing the serum solids content of the mix had no effect on the final phenol value when the mix was pasteurized at 150° F. for 30 minutes.

6. Storing pasteurized, unflavored and uncolored ice cream mix at 40° F. for a period of two weeks did not appreciably alter the phenol value.

7. Storing unflavored and uncolored ice cream at − 20° F. over a period of twelve weeks resulted in marked decreases in the phenol values of all the samples except those made from mix samples taken 20, 25 and 30 minutes after reaching 150° F. The samples heated for a period of time less than 20 minutes, however, still showed sufficient phenol content for them to be considered underpasteurized.

8. Pure vanilla had no effect on the phenol value while artificial and imitation vanilla compounds caused a slight increase and coumarin and vanillin caused appreciable increases in the phenol value of the mix.

9. Flavoring extracts (other than vanilla) added to the mix did not appreciably alter the phenol value, while the addition of "Cold-Pak" fruits increased the phenol value.

10. The addition of coloring material to ice cream mix in many instances affected the phenol value unless the coloring matter was precipitated with the protein upon the addition of the lead acetate. This, however, was not always possible.

11. Tests run on commercial samples of ice cream mix gave indication of complete pasteurization in all but one case.

CONCLUSIONS

Within certain limits the Scharer Test can be applied as a check on the efficiency of the pasteurization of ice cream mix.

The necessity of establishing standard phenol values for complete pasteurization at various temperatures was definitely shown. Pasteurizing ice cream mix at temperatures of 147° F. or below may result in variable phenol values. This is due to the fact that variation in the heat treatment previously given the dairy products from which the mix is made will cause variation in the amount of phosphatase that survives pasteurization. It is evident that the heat treatment, when pasteurizing mix at temperatures of 147° F. or below, is not sufficient to result in any appreciable inactivation of the enzyme. Apparently the additional solids in the ice cream mix act as a protective agent for the phosphatase enzyme so that more heat is necessary to destroy the enzyme than in the case of milk pasteurization. The possibility that the mix solids act as a protective agent against destruction of bacteria by heat might follow in the same line of reasoning so that the question arises as to whether temperatures of 147° F. or below are sufficient for the proper pasteurization of ice cream mix.

Since coloring and flavoring material affect the accuracy of the phosphatase test, it is evident that some modification will have to be made in the test before it can be used on any unknown sample of ice cream.

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A STUDY OF FACTORS CONCERNING HERD MANAGEMENT AND HERD PRODUCTION

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It is becoming a recognized fact that too often in the past breeders have secured official production records on their cows with the primary object of using those records as a means of selling bull calves and surplus breeding stock We have not studied the individual records on our cows nor have we analyzed them sufficiently with the thought of improving our own herds nor have we as individual breeders concerned ourselves enough with the improving of our respective breeds

Every official production record on a registered cow of any breed possesses many values. First, it indicates the economic worth of the cow as a producing unit in the herd Then a production record on an animal furnishes some information as to her transmitting ability to her sons and daughters Furthermore, the record of the cow assists in proving both her sire and her dam. Likewise the butterfat percentage for the lactation and the persistency of production are valuable for study with the view of maintaining or increasing the average test of the herd and in improving persistency Also, good production records possess considerable advertising and publicity value and such records add to the sale price of both the cow and her progeny. In addition to the individual records, the information each year on the herd average including the average fat percentage of the herd, breeding efficiency, length of dry periods, services required per cow and the average age of the herd are all of value to the breeder who will study and use such data in trying to do a really constructive work of. breeding better dairy cattle.

Herd Improvement Registry records are particularly valuable for herd improvement, due to the fact that every cow in the herd is included and in addition information is available on the herd average each year. To January 1st, 1938, a total of 1046 Jersey herds had completed a year's work of official Herd Improvement Registry testing. These herds included a total of 27,364 cows, of which number 12,274 have finished complete lactation records. The individual lactation records completed by these cows and the information on the herd averages when summarized and tabulated show facts that are of interest and value to every breeder of dairy cattle. It is the object of this paper to present some of the material summarized from these Herd Test records and to attempt to draw such conclusions as seem evident from the data. The paper will be observed to lack continuity in that it does not deal entirely with one subject but instead many different

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observations that seemed to be of interest and of value have been summarized and are presented.

Quite often the term, breeding efficiency, has been used to denote the number of calves per cow, per year, and formulas have been published illustrating how the breeding efficiency of a herd may be computed on this basis. However, in this paper liberty has been taken to use the term, breeding efficiency, as indicating the fraction of the year which a cow is in milk or inversely the percentage of the herd dry during the year. This of course is definitely related to the number of calves dropped during the year although persistency of production may also influence it.

In observing the data on the herd averages as completed each month, over a period of years, it seemed evident that breeding efficiency and the average production of the herd were directly related. On the certificate which a breeder receives at the end of the Herd Test year and also in the annual record volumes, two figures are given which indicate in a large measure how efficiently the herd has been operating from a breeding and calving standpoint. One figure shows the average number of cows in the herd and the other figure shows the average number of cows in milk throughout the year. The difference between these two figures represents the average number of days that each cow was dry during the year. Table 1 shows the relationship between the breeding efficiency and herd averages of the 1046 herds that had completed a year's work of herd testing prior to January 1st, 1938.

TABLE 1

Effect of breeding efficiency on herd average

Production divisions	Number of herds	Percentage of herd dry	Average number of days each cow was dry
Below 250 lbs. fat	40	27.6	101
250 to 299 lbs.	119	18.8	69
300 to 349 lbs.	248	15.9	58
350 to 399 lbs.	344	13.3	49
400 to 449 lbs.	190	11.5	42
450 to 499 lbs.	60	10.5	38
500 lbs. and over	45	10.4	38
Totals (All herds)	1046	14.5	53

The coefficients of correlation existing between the herd averages of the 1046 herds and the percentage of dry cows in each herd was determined and found to be $-.57 \pm .014$. This correlation is sufficiently high to be significant and together with the data in the table indicates that the percentage of dry cows in the herds on Herd Test is inversely proportional to the herd average. In fact it seems that the low average yield of many herds is not due entirely to low inherited producing ability but that perhaps breeding troubles and management were also largely responsible. The carrying

TABLE 2

Frequency table showing percentage of cows dry in herds of different levels of production

Production divisions	Percentage of herd dry									
	Under 5%	5 to 9%	10 to 14%	15 to 19%	20 to 24%	25 to 29%	30 to 34%	35 to 39%	40% and over	Totals
Under 250 lbs.	0	0	3	3	11	7	9	3	4	40
250 to 299 lbs.	0	8	26	38	30	9	5	2	1	119
300 to 349 lbs.	0	17	106	78	35	9	1	2	0	248
350 to 399 lbs.	9	67	173	77	13	4	1	0	0	344
400 to 449 lbs.	5	58	88	39	0	0	0	0	0	190
450 to 499 lbs.	5	21	28	6	0	0	0	0	0	60
500 lbs. and over	4	17	21	3	0	0	0	0	0	45
All herds	23	188	445	244	89	29	16	7	5	1046

of old cows, long past their prime in production, in the hope of getting another calf or two is customary in some herds. This, however, can hardly be responsible for the high percentage of dry cows in the low producing herds for such brood cows are usually carried only in the better herds.

The average percentage of dry cows in all the herds was found to be 14 48 per cent. It may be that this percentage is somewhat too low for quite often when entering a herd on test for the first time, the owner starts the work when the majority of the cows in the herd have just freshened and in view of this, the percentage of dry cows during the first year's work may be lower than usual. It was found that a total of 368 herds had completed three or more years' work of herd testing and it was observed that the percentage of dry cows in these 368 herds during the third year's work was 15.51 per cent or each cow was dry an average of 57 days or for about eight weeks during the year. This average percentage is a good yardstick by which any breeder may compare the breeding efficiency of his own herd with that of other purebred herds.

Table 2 shows a frequency chart illustrating in a different manner just how important breeding efficiency is to average herd production. In examining this table, it will be observed that not one of the 146 herds with a percentage of dry cows of twenty per cent or more has completed a herd average of above 400 pounds of butterfat and only eighteen of the 146 herds carrying twenty per cent or more dry cows have herd averages of above 350 pounds of butterfat. Furthermore, of the 40 herds averaging under 250 pounds, 16 carried 30 per cent or more of the cows dry during the year

The size of the herd also affects breeding efficiency and from the data in Table 3, it appears that it is easier to maintain the breeding efficiency of a small herd than it is in the case of a larger one. This result was not unexpected for it is realized that most of the small herds are owner operated, whereas large herds are usually cared for entirely by hired labor. Furthermore, disease control becomes increasingly difficult as the size of the herd increases.

TABLE 3

Breeding efficiency as affected by size of herd

Size of herd	Number of herds	Percentage of herd dry	Herd averages (lbs. fat)
Less than 10 cows	228	12.7	391.46
10 to 19 cows	472	14.3	364 32
20 to 29 cows	209	15 1	361 47
30 to 39 cows	60	16.0	369.63
40 cows and over	77	17.7	338.77
All herds	1046	14.5	368.03

It is common knowledge that any herd regardless of the herd average will show a considerable range in the producing ability of the various members of the herd. To determine just what this range was with the Jersey herds doing herd testing, a tabulation was made of these herds. The accompanying graph (Figure 1) shows the range in yield of the cows in these herds.

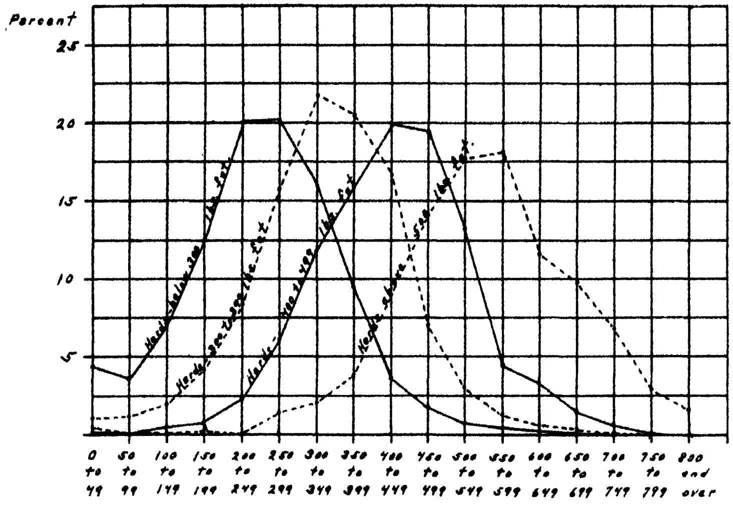

FIG. 1. Illustrating range in yield of cows in herds of varying levels of production.

It will be observed that herds of widely different levels of production show very similar frequency curves in the distribution of the cows on the basis of production. In other words, even those herds averaging over 400 pounds of butterfat usually contain cows with a production very much lower than the average. The chart shows that the amount of variation in production in the high producing herds is as great as in herds of lower levels of production and demonstrates that regardless of the herd average, rigid culling may usually be practiced with advantageous results.

For years all of the Breed Associations have published average production figures of cows on Register of Merit and Advanced Registry test. Such averages however, are based on selected animals and such cows have usually been given better than average attention in regard to feeding and management during the making of the records. Furthermore, the Register of Merit and Advanced Registry requirements have automatically eliminated from such data the production records of low producing animals. In view of this, the information on the averages of the individual Herd Test lactation records is especially pertinent at this time. These records have been summarized depending upon the length of the lactations and the ages of the cows. The summaries are presented in Table 4.

TABLE 4

Herd improvement registry lactation record averages

Length (days)	No. of records	Aver. milk	Aver. fat per cent	Aver. fat	No. of records	Aver. milk	Aver. fat per cent	Aver. fat
	Yearlings				Junior-two-year-olds			
180 or less	17	1608	5.111	82.18	41	1966	5.193	102.10
181 to 210	10	2602	5.108	132.90	18	3148	5.584	175.78
211 to 240	8	2980	5.373	160.13	39	3709	5.173	191.87
241 to 270	59	4220	5.308	223.98	132	4624	5.302	245.16
271 to 305	147	5043	5.347	269.64	420	5511	5.340	294.27
306 to 335	150	5796	5.419	314.07	482	6460	5.299	342.29
336 to 365	261	6479	5.415	350.82	760	7168	5.412	387.95
Totals	652	5564	5.390	299.90	1892	6220	5.359	333.30
	Senior-two-year-olds				Junior-three-year-olds			
180 or less	21	2157	5.170	111.52	24	2285	5.186	118.50
181 to 210	9	3006	5.086	152.89	25	3812	5.538	211.12
211 to 240	35	3899	5.245	204.49	58	4544	5.333	242.34
241 to 270	75	4842	5.262	254.81	189	5432	5.338	289.94
271 to 305	217	5629	5.390	303.38	374	6152	5.335	328.22
306 to 335	203	6688	5.486	366.90	292	7175	5.326	382.15
336 to 365	358	7460	5.446	406.29	436	7815	5.413	423.01
Totals	918	6342	5.424	343.96	1398	6612	5.363	354.61
	Senior-three-year-olds				Junior-four-year olds			
180 or less	19	2216	5.246	116.26	18	2279	5.302	120.83
181 to 210	7	3277	5.358	175.57	13	4170	5.340	222.69
211 to 240	35	4565	5.201	237.43	44	4577	5.122	234.45
241 to 270	93	5418	5.326	288.57	113	5706	5.306	302.75
271 to 305	243	6374	5.390	343.59	292	6646	5.374	357.13
306 to 335	218	7503	5.388	404.24	262	7777	5.365	417.22
336 to 365	300	8142	5.516	449.11	341	8544	5.402	461.57
Totals	915	6946	5.427	376.98	1083	7233	5.369	388.35
	Senior-four-year-olds				Five-year-olds			
180 or less	19	2184	5.000	109.21	40	2422	5.186	125.60
181 to 210	12	4217	5.059	213.33	23	4078	5.161	210.48
211 to 240	26	4511	5.280	238.19	45	4778	5.171	247.09
241 to 270	63	5586	5.273	294.56	147	5922	5.319	314.97
271 to 305	206	6620	5.330	352.84	358	6952	5.281	367.11
306 to 335	196	7777	5.303	412.41	316	8111	5.316	431.19
336 to 365	260	9053	5.336	483.06	442	8994	5.367	482.74
Totals	782	7421	5.316	394.50	1371	7515	5.322	399.96

TABLE 4—(*Continued*)

Length (days)	No. of records	Aver. milk	Aver. fat per cent	Aver. fat	No. of records	Aver. milk	Aver. fat per cent	Aver. fat
	Six-year-olds				Seven-year-olds			
180 or less	28	2537	4.988	126.54	24	2608	4.991	130.17
181 to 210	21	3825	5.407	206.81	16	3942	5.248	206.88
211 to 240	36	4908	5.041	247.42	28	5040	5.077	255.86
241 to 270	104	6355	5.197	330.27	74	6111	5.124	313.11
271 to 305	270	6987	5.201	363.41	183	7129	5.200	370.71
306 to 335	230	8110	5.258	426.43	172	8149	5.156	420.20
336 to 365	364	9069	5.297	480.38	264	8983	5.267	473.09
Totals	1053	7637	5.250	400.95	761	7617	5.206	396.56
	Eight-year-olds				Nine-year-olds			
180 or less	20	2349	4.887	114.80	18	2011	4.777	96.06
181 to 210	13	3239	5.220	169.08	5	3723	5.017	186.80
211 to 240	20	4824	4.992	240.80	14	4913	5.292	260.00
241 to 270	50	5770	5.036	290.56	49	5591	4.960	277.29
271 to 305	127	6931	5.150	356.92	75	6987	5.235	365.76
306 to 335	98	8248	5.196	428.57	69	7813	5.106	398.93
336 to 365	205	8761	5.341	467.91	137	8445	5.287	446.47
Totals	533	7427	5.231	388.53	367	7133	5.196	370.61
	Ten-year-olds				Eleven-year-olds			
180 or less	11	1462	4.583	67.00	7	2132	4.818	102.71
181 to 210	2	4406	5.050	222.50	6	3245	4.859	157.67
211 to 240	7	4483	5.006	224.43	5	5079	4.733	240.40
241 to 270	25	5683	4.965	282.16	13	6595	5.013	330.62
271 to 305	60	7008	5.146	360.63	27	7176	5.225	374.93
306 to 335	50	7706	5.106	393.44	20	7537	5.084	383.20
336 to 365	77	8643	5.260	454.66	64	8615	5.239	451.31
Totals	232	7196	5.159	371.23	142	7334	5.169	379.13
	Twelve-year-olds and over				Summary (all ages)			
180 or less	17	2022	4.524	91.47	324	2197	5.051	110.97
181 to 210	6	3391	4.502	152.67	186	3652	5.265	192.28
211 to 240	7	5234	4.976	260.43	407	4517	5.170	233.51
241 to 270	17	5265	4.830	254.29	1203	5497	5.243	288.20
271 to 305	31	6153	5.024	309.19	3030	6376	5.299	337.88
306 to 335	30	7667	5.138	393.93	2788	7381	5.305	391.58
336 to 365	67	8102	5.073	410.99	4336	8138	5.372	437.19
Totals	175	6539	5.030	328.88	12274	6927	5.322	368.63

In examining the summary of this table, it is significant to observe that the average butterfat percentage of the cows that have completed lactation records in the Herd Improvement Registry of 336 to 365 days is almost identical with the previously published average of the breed based on cows that have completed yearly Register of Merit records. It is also significant to point out that the average production of the 4336 cows of all ages with complete lactation records of 336 to 365 days in length is 437.19 pounds of butterfat per cow, whereas the average yield per cow of 30,351 cows all ages that have completed yearly Register of Merit records from 1903 to 1937 inclusive, is 488.31 pounds of butterfat per cow. It would appear that there is not the extreme difference between Herd Improvement Registry lactation records and Register of Merit records as has often been assumed in the past. The grouping of the records by length of lactations, furnishes information regarding persistency of production. Turner (1) has stated that persistency of production tends to decline gradually with age. This fact seems to be substantiated by the summaries of the lactation records as presented in Table 5.

TABLE 5

Effect of age on length of individual lactation records

	Young cows		Mature cows		Old cows	
Length of lactations	Yearlings Jr. 2-year-olds Sr. 2-year-olds Jr. 3-year-olds Sr. 3-year-olds Jr. 4-year-olds Sr. 4-year-olds		5-year-olds 6-year-olds 7-year-olds 8-year-olds		9-year-olds 10-year-olds 11-year-olds 12-and-over	
	Number	Per cent	Number	Per cent	Number	Per cent
180 days or less	159	2.1	112	3.0	53	5.8
181 to 210 days	94	1.2	73	2.0	19	2.1
211 to 240 days	245	3.2	129	3.5	33	3.6
241 to 270 days	724	9.5	375	10.1	104	11.4
271 to 305 days	1899	24.9	938	25.2	193	21.1
306 to 335 days	1803	23.6	816	21.9	169	18.5
336 to 365 days	2716	35.6	1275	34.3	345	37.7
All lengths	7640		3718		916	

In examining this table, it will be seen that 84 per cent of the cows under five years of age showed complete lactation records of longer than 270 days. Of the cows from five to eight years of age, inclusive, 81 per cent showed complete lactation records of more than 270 days, whereas with the old cows past nine years of age, only 77 per cent had lactation records of more than nine months.

During the calendar year 1937, a total of 297 herds completed a year's work of herd testing. These herds were studied to learn several facts concerning herd management, such as culling, average age of cows in the herds, the number and ages of first calf heifers and the production of these first calf heifers. It was found that at the beginning of the year's work of each herd, there were a total of 5864 cows in the herds that had previously freshened in their lifetimes. The average age of these 5864 cows at the beginning of the year's work was five years two months. During the year in these 297 herds, there were a total of 1582 new heifers that came into milk. It was interesting to observe the ages at which these heifers freshened and this is summarized in Table 6.

TABLE 6

Ages of heifers calving for first time during year

Age groups	Number	Age groups	Number	Age groups	Number
1-2	1	2-3	122	3-4	4
1-3	1	2-4	124	3-5	2
1-4	0	2-5	103	3-6	3
1-5	5	2-6	64	3-7	2
1-6	6	2-7	67	3-8	2
1-7	8	2-8	45	3-9	0
1-8	30	2-9	27	3-10	0
1-9	35	2-10	34	3-11	1
1-10	77	2-11	15	4-0	0
1-11	186	3-0	24	4-1	1
2-0	210	3-1	7	4-2	1
2-1	208	3-2	3		
2-2	161	3-3	3	Totals	1582

The average age at which these 1582 heifers calved was 26.49 months. Plum and Lush (2), reporting on a smaller number of animals in Iowa Cow Testing Associations, found the mean age for first calf Jersey heifers to be 25.5 months. Of course it is realized that many heifers required more than one service, and consequently the average age at first calving of 26.49 months is undoubtedly higher than what most Jersey breeders consider the ideal age for first freshening.

Breeders have often expressed the thought that the heifers calving during the year were a distinct handicap to the herd average. However, such is not the case. The herd average of any herd is based on the average number of cows in the herd and not on the average number of cows in milk. In most instances, the heifers calving for the first time during the year are milked until the year's work is completed and thus they have no dry period charged against them. On a calendar year basis, there were 723.55 first calf heifers in the herd and the average number of heifers in milk during the year was 701.37. The average butterfat yield of these first calf heifers, computed in the usual manner of figuring herd averages, is 391.16 pounds of butterfat. This is in excess of the average yield (370.69 lbs.) of all of the herds that have completed a year's work of herd testing in 1937. Consequently, it is evident that heifers calving for the first time during the year help the herd average rather than handicap it.

During 1937, there were purchased and added to these 297 herds, a total of 522 cows of milking age The ages of these cows purchased are shown in Table 7

TABLE 7

Ages of cows added to the herds during year

Age groups	Number of cows added	Per cent added
Yearlings	7	1.34
2 year olds	79	15.13
3 year-olds	124	23.76
4 year olds	103	19.73
5 year olds	75	14.37
6-year olds	55	10.54
7 year olds	35	6.71
8 year-olds	13	2 49
9 year olds	14	2.68
10 year olds	8	1.53
11 year olds	3	.58
12 and over	6	1.15
Totals	522	

This table is of interest in illustrating the ages of cows which most breeders desire to purchase as additions to the herd. Likewise the ages of the cows culled or removed from the herds during the year is of equal interest. During the year a total of 1452 cows were removed from the 297 herds for one reason or another In most instances, the supervisors do not report the cause of removal and hence it is not known whether these cows were culled because of low production, breeding difficulties or whether they were sold as surplus breeding stock. However, the ages of the cows that were culled during the year is of concern and this data has been summarized in Table 8. It will be noted from the information in this table that relatively few of the cows removed from the herds have been culled due to old

TABLE 8

Ages of cows removed from herds during year

Age groups of cows in herds	Number of cows in each age group	Number of cows sold or culled during year	Percentage of each age group sold or culled
Yearlings	413	43	10.41
2-year-olds	2321	313	13.49
3-year-olds	1395	238	17.06
4-year olds	1102	202	18.33
5-year-olds	792	163	20.58
6-year-olds	620	145	23.39
7-year-olds	439	78	17.77
8-year-olds	323	91	28.17
9-year-olds	180	51	28.33
10-year-olds	152	47	30.92
11-year-olds	107	35	32.71
12 years and over	124	46	37.10
Totals	7968	1452	18.23

age, or died as a result of advanced age, for over 54 per cent of all the cows removed from all the herds during the year were under five years of age.

In compiling the foregoing data, it was noted that with Jersey herds on Herd Improvement Registry test, relatively few cows were used as nurse animals. Sixty-eight herds were on test in the northeastern section of the country and of this group only twenty-three used a total of thirty-eight cows as nurse animals during the year. There were 122 herds on test in the north central states and of this number thirty-four herds used a total of sixty-three nurse cows. There were sixty-three herds on test in the southern states and twenty-two of these herds used a total of sixty-seven nurse cows. Forty-four herds were on test in the Rocky Mountain and Pacific states and six of these herds used a total of just six nurse cows during the year. Apparently, only a small percentage of registered calves are raised with the use of nurse animals.

SUMMARY

It is difficult to summarize all of the data presented but such conclusions as can easily be drawn are as follows:

1. The percentage of dry cows in the herd during the year is one of the main factors affecting the herd average.
2. The average percentage of dry cows in all herds is approximately fifteen per cent.
3. Breeding efficiency and size of the herd are inversely related.
4. Culling can always be practiced with advantageous results regardless of the herd average.
5. Persistency of production tends to decline slightly with age.
6. Herd Test lactation records of ten months or more in length average between ten and twelve per cent less than Register of Merit records of similar length.
7. The average age at which Jersey heifers freshen is two years two and one-half months.
8. The average age of all the cows in the herds that have freshened in their lifetimes is five years two months.
9. Heifers freshening for the first time during the year do not handicap the herd average.
10. More three- and four-year-old cows are added to the herds than cows of any other age.
11. Over 54 per cent of all the cows removed from the herds during the year were under five years of age.
12. Relatively few cows are used in registered herds as nurse animals.

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THE CHEMICAL COMPOSITION OF THE BLOOD OF THE DAIRY GOAT

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The adaptability of the dairy goat for many types of experimental work is becoming recognized. For the study of the biochemistry of milk secretion and the influence of the hormones upon the growth of the udder and the stimulation of milk secretion, the small size yet easily obtainable milk yield of the dairy goat makes it a most suitable animal for preliminary experiments.

In the use of the dairy goat for experimental purposes it is desirable to establish norms or standards with which the experimental conditions may be compared with studies of the precursors of milk.

Fish and Hayden (1) and Hayden and Fish (2) reported values for certain constituents of the blood of the normal and castrated male goat. For the purpose of studying carbohydrate metabolism, Cutler (3) gave figures on the glucose and inorganic phosphorus content in the blood of normal goats and during various pathological conditions. At about the same time Lintzel (4) published material concerning milk precursors which included data on the blood of lactating goats. Included in his data are the figures for both arterial and mammary venous blood.

In this communication average values for the more important constituents of arterial and venous blood of dry and lactating dairy goats are reported as tentative normal standards.

The data to be presented were obtained in the course of a series of experiments having as their objective the determination of certain precursors of milk (5, 55, 59, 63).

The blood used for analysis was taken from the carotid artery, which is recognized as typical arterial blood, and from the mammary vein. Graham, Turner and Gomez (6) described a method of preparing the artery by exteriorization for the purpose of obtaining arterial blood. The mammary venous blood was taken to represent typical blood from the udder since all peripheral veins were previously ligated. Precautions followed in drawing the blood samples were given in the publication of Graham, Houchin and Turner (5).

The analytical methods used were applied to both whole blood and plasma. The blood was prevented from clotting by the use of potassium oxalate. Plasma and cells were separated by centrifugation. Where the

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procedures necessitated the use of filtrates, *i.e.*, N–P–N and amino acid, sufficient quantities were prepared to take care of all determinations. The analyses were run until duplicates checked within one per cent.

Total nitrogen was determined by the Kjeldahl method. Urea was determined on whole blood and plasma by the urease, manometric method of Van Slyke (7); in case sufficient blood was not available for the former method, the determination was made on the Folin-Wu filtrate by the method of Van Slyke (8). Amino nitrogen was determined by the Danielson (9) method.

Amide nitrogen was determined by digesting the Folin-Wu precipitate to break off the amide particle according to the method described by Bliss (10), aerated, then nitrogen determined manometrically using sodium hypobromite. Non-protein nitrogen was determined on the Folin-Wu filtrate by means of the manometric micro-Kjeldahl method described by Van Slyke (11).

The fibrin of the plasma was diluted with isotonic NaCl solution and precipitated with calcium chloride, Howe (12). Micro-Kjeldahl digestion and hypobromite manometric determination were then made for nitrogen (11).

Albumin nitrogen plus non-protein nitrogen in plasma was fractionated from the globulin by Howe's method (12) then nitrogen determined by the Kjeldahl method. The globulin plus fibrin fraction was calculated by subtracting the concentration of albumin plus non-protein nitrogen from the total plasma nitrogen. To find the albumin nitrogen, the value obtained for non-protein nitrogen was subtracted from the albumin plus non-protein nitrogen fraction. The fibrin value was subtracted from the globulin plus fibrin fraction in calculating the globulin nitrogen content.

Blood glucose, without other reducing materials, was determined by use of the Shaffer, Hartman and Symogyi (13, 14) iodometric titration method. In a majority of cases the colorimetric method of Mendel and Goldscheider (15) was used to determine lactic acid. To verify results by the above method, some determinations were made by the von Furth-Charness method (16) as modified by West (17).

The simultaneous manometric method of Van Slyke and Neill (18) was used for oxygen and carbon dioxide, oxalate was used as anticoagulant and sodium floride to prevent glycolysis and bacterial action. The hemoglobin content was found by the method of Newcomer (19) and cell volume by the use of hematocrit tubes.

Both the inorganic and organic phosphates were determined by the method of Whitehorn (20) and Benedict (21).

The data on the composition of the whole blood and plasma of both lactating and non-lactating goats were combined (Table 1). The samples from lactating animals were taken at increasing intervals after milking. Only a few of the samples were analyzed for all of the above mentioned constituents. In addition to the average value, the variation in each constituent is

TABLE 1

Chemical composition of goat blood

Constituents	Arterial				Venous			
	No. of samples	Ave. conc.	Range		No. of samples	Ave. conc.	Range	
			High	Low			High	Low
			Whole blood					
Total nitrogen (mg. %)	32	2505.04	2947.30	2142.70	32	2507.00	2850.50	2133.90
Urea nitrogen (mg. %)	32	22.31	33.88	14.53	32	23.16	36.72	14.80
Amino nitrogen (mg. %)	24	9.06	11.83	7.19	23	8.37	10.45	6.28
Amide nitrogen (mg. %)	6	128.40	152.70	119.57	6	119.31	141.40	103.53
Non-protein nitrogen (mg. %)	19	48.52	63.20	35.90	19	48.50	75.90	34.67
Sugar (mg. %)	28	59.07	77.37	33.93	27	48.12	72.35	34.88
Lactic acid (mg. %)	28	53.88	134.30	21.29	31	50.54	100.00	23.56
Total phosphorus (mg. %)	19	22.72	33.48	13.37	19	23.21	32.48	18.02
Inorganic phosphorus (mg. %)	15	7.69	11.91	5.76	14	6.91	8.72	5.63
Oxygen (vol. %)	25	12.80	15.41	9.56	25	8.48	12.60	4.62
Carbon dioxide (vol. %)	25	46.74	56.91	36.65	25	53.31	62.02	43.92
Hemoglobin (gm. %)	16	9.26	14.40	5.94	15	9.41	12.00	8.02
Cell (vol. %)	7	28.84	36.30	24.09	7	29.21	38.60	24.56
			Plasma (gm. %)					
Total nitrogen	26	1228.20	1708.80	1043.70	26	1204.45	1518.70	1090.00
Alb. + NPN	23	468.98	612.80	298.90	24	472.84	637.60	277.60
Globulin + fibrin	23	759.22	1145.10	597.10	24	731.61	933.70	544.40
Urea nitrogen	4	20.23	26.86	17.16	4	19.69	26.98	16.90
Amino nitrogen	6	7.70	9.82	6.06	6	7.19	9.27	5.63
Amide nitrogen	5	96.34	103.20	81.24	8	69.39	98.35	41.64
Non-protein nitrogen	6	38.33	43.20	32.66	7	39.28	47.00	28.82
Fibrin nitrogen	6	83.48	113.03	66.38	6	100.88	124.90	81.17

TABLE 2

The relation of the blood from the lactating and non-lactating goat

Constituents	Arterial				Venous			
	Lactating		Non-lactating		Lactating		Non-lactating	
	No. of samples	Ave. conc.	No. of samples	Ave. conc.	No. of samples	Ave. conc.	No. of samples	Ave. conc.
Whole blood								
Total nitrogen (mg. %)	23	2511.10	9	2490.00	23	2540.60	9	2421.20
Urea nitrogen (mg. %)	22	23.07	10	21.76	22	23.64	10	22.12
Amino nitrogen (mg. %)	16	9.12	8	8.93	15	8.47	8	8.17
Amide nitrogen (mg. %)	3	135.23	3	121.50	3	122.40	3	116.20
Non-protein nitrogen (mg. %)	11	51.21	8	44.76	10	48.99	9	47.97
Sugar (mg. %)	20	59.87	8	57.08	20	45.27	7	56.28
Lactic acid (mg. %)	20	58.77	6	37.35	21	50.08	9	43.61
Total phosphorus (mg. %)	17	23.55	2	20.63	16	23.60	2	20.85
Inorganic phosphorus (mg. %)	15	7.69			13	6.91		
Oxygen (vol. %)	22	12.64	3	14.15	22	7.24	3	9.14
CO_2 (vol. %)	22	47.32	3	42.45	22	53.89	3	49.00
Hemoglobin (gm. %)	9	8.73	7	9.90	10	9.34	5	9.27
Cell (vol. %)	5	28.30	2	30.20	5	27.89	2	31.68
Plasma (mg. %)								
Total nitrogen	12	1236.60	14	1220.90	13	1193.20	13	1215.50
Alb. + NPN	11	478.00	11	453.30	12	459.10	12	486.80
Globulin + fibrin	11	758.60	11	767.60	12	734.10	12	729.00
Urea nitrogen	2	22.80	2	17.68	2	21.94	2	17.43
Amino nitrogen	3	8.30	3	7.08	3	7.90	3	6.45
Amide nitrogen	3	100.50	3	82.40	4	80.10	4	57.70
Non-protein nitrogen	2	40.17	4	37.40	3	42.06	4	36.70
Fibrin	5	86.31	1	69.33	4	103.97	2	94.70

indicated by the presentation of the range. Most of the samples came from non-pregnant goats during their lactating and subsequent dry period.

Comparison of the arterial and venous (mammary) blood show only a few significant differences, *i.e.*, amide nitrogen, sugar, lactic acid, oxygen, carbon dioxide and fibrin. The ranges of both are usually comparable. Some constituents showed higher arterial blood values than the corresponding venous values although the averages may be similar. As should be expected the range levels of the oxygen are much higher in the arterial blood than in venous blood. On the other hand, the carbon dioxide in the arterial blood is low and in the venous blood high.

The range for total nitrogen showed the greatest absolute variation. However, the per cent variation of the lactic acid values was greater than that of any other constituent. Many of the constituents have range differences that show no large variation.

A majority of the experiments were on lactating goats. At the latter part of the experiment some determinations were made on dry animals. Only averages were presented to show the relation between the lactating and non-lactating goats (Table 2) Some constituents show considerable difference between the lactating and non-lactating condition. The oxygen content was found to be higher in the non-lactating animals, while the carbon

TABLE 3

Relation of goat blood to other domestic animals

	Total plasma N	Urea N	NPN	Amino N	Sugar	Inorganic phosphorus	Hemoglobin	Cell
	mg. %	*mg. %*	*mg %*	*mg. %*	*mg %*	*mg. %*	*gm. %*	*vol. %*
Cattle	1177.6 (2)	12.2 (2)	30.0 (27)	7.19 (36)	53.0 (39)	5.7 (2)	10.90 (52)	34.3 (36)
Horse	1296.0 (23)	18.7 (21)	25.8 (31)		109.0 (31)			33.0 (51)
Sheep	1054.4 (22)	13.0 (26)	28.5 (33)	4.76 (37)	63.90 (40)	7.1 (44)		
Dog	1131.2 (22)	11.7 (28)	30.8 (28)	6.70 (28)	82.00 (28)	3.5 (45)	14.11 (47)	47.7 (47)
Rabbit	1072.0 (23)	13.0 (26)	31.0 (32)		124.0 (43)	4.5 (46)		
Swine	1216.0 (22)	17.3 (29)	31.4 (34)		128.0 (34)		11.95 (48)	47.8 (48)
Rat	1040 (23)	15.6 (29)	42.0 (35)		122 (42)			48.0 (40)
Hen		4.53 (25)	38.5 (25)		212 (25)	3.96 (2)		
Cat	1347.2 (22)	30.3 (26)	52.6 (32)					
Man	1120 (24)	17.1 (30)	35.6 (30)	6.40 (38)	112.0 (30)			45.6 (50)
Goat*	1128	22.31	48.52	9.60	59.1	7.70	9.26	28.8

Numbers in parenthesis denote reference from which taken.
* Data from this paper.

dioxide value was lower and showed a lower R. Q. Lactic acid was reversed in regard to A–V difference in the lactating and non-lactating animal, being positive in those lactating. The venous blood of the lactating goat had less glucose than that of the non-lactating goat, while the arterial values were virtually the same. Values for other constituents did not show much variation between the lactating and non-lactating condition.

Goat's blood has been found to be relatively similar to the blood of other domestic animals in regard to the concentration of its constituents (Table 3). A tabulation of the composition of the blood of a number of domestic and laboratory animals has been prepared for comparison with average goat blood as presented in Table 1.

For most constituents the value for the goat is intermediate in comparison with other animals. The cell volume, however, is considerably lower than that of most animals included. As would be expected the hemoglobin value for the goat is lower than that of other animals but not to as large extent as the cell volume. In a few cases such as amino acid and inorganic phosphorus, the goat blood shows a higher value than any other animal. From these data it would seem that goat blood is not a peculiar type but is in most respects similar in composition to that of other species reported.

DISCUSSION

During the past few years, this department has been engaged in a study of the precursors of milk using dairy goats as experimental animals. This work was initiated following the development by Herrick and Baldes (53) of a technique for the measurement of the blood flow in normal intact animals. If this method could be adapted to the measurement of the arterial or venous blood passing through the mammary gland, the milk precursors could be studied quantitatively and the efficiency of the udder as a converter could be determined.

The classical experiments of Kaufman and Magne (54) which have been confirmed abundantly indicate that considerable quantities of glucose are removed from the blood during its passage through the mammary gland. These observations have led to the opinion that glucose is the precursor of lactose. However, when the blood flow measurements were combined with the analyses of arterial and venous glucose, it was observed that considerably more lactose was being secreted in the milk than could be accounted for by the removal of glucose from the blood, Graham (55). It appeared necessary to examine the blood for other carbohydrate-forming compounds which might be taken up by the udder. It was subsequently observed that considerable amounts of lactic acid were also removed from the blood in its passage through the gland. In further experiments it was calculated that 85 per cent of the lactose formed during the period could be accounted for theoretically from the glucose and lactic acid removed from the blood. It is interesting to note in passing that considerable quantities of lactic acid have since been observed by Shaw and Petersen (56) to be withdrawn from the blood of the dairy cow during its passage through the lactating udder. In further confirmation of the above evidence that lactic acid is a precursor of lactose, Petersen and Shaw (57) showed that lactose could be synthesized *in vitro* from a substrate containing glucose and lactic acid to which macerated mammary gland tissue from lactating cows was added.

Since the experiments of Cary (58) and others showed that amino acids are removed from the blood during its passage through the lactating mammary gland, it has been generally assumed that the amino acids were the chief, if not the sole precursors of milk protein. Our recent studies have shown quite definitely that insufficient amino acid is taken up by the active gland to account for more than a small part of the milk protein secreted (59). Shaw and Petersen (60) have recently confirmed this observation and have claimed further that not more than 35 per cent of the milk proteins could be formed from this source (61). Further light was thrown on the nitrogen metabolism of the lactating mammary gland by our observation that urea was being produced in amounts equivalent to the nitrogen contained in the amino acids taken up by the udder. Recently the presence of arginase, an enzyme capable of synthesizing urea, was observed to be present in the lactating but not in the non-lactating mammary gland (62).

From these observations it would appear that the possibility exists that certain amino acids may be deaminized and converted into lactic acid which would be a further source of carbohydrates. Consequently, the precursors of milk protein must be sought in other nitrogenous compounds of the blood. Further study revealed that in addition to amino N, globulin and unknown fractions of the non-protein nitrogen of the blood were being taken up by the active mammary gland (50). Work is now in progress having as its object the determination of the particular fraction of the nitrogen partition of the blood utilized by the mammary gland.

In the course of the investigations discussed above, a number of the constituents of goat's blood were determined. As the results were presented chiefly as arterial-venous differences, it seemed desirable to bring together the average values for arterial and venous goat blood obtained from this work. It is hoped that these values will be useful in the development of normal standards of dairy goat blood.

SUMMARY AND CONCLUSIONS

Data were presented on the range and average composition of certain constituents of the whole blood and plasma of dairy goats. A comparison of the arterial and venous blood of lactating and non-lactating animals was made.

For purposes of comparison, a tabulation of comparative data on the composition of the blood of other species was presented. No significant differences in the goat plasma were noted in comparison with other mammals. It will be seen that the cell volume of the goat is unusually low probably due to the small size of the red blood corpuscle.

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STUDIES ON THE COMPOSITION OF BOVINE BLOOD AS INFLUENCED BY GESTATION, LACTATION, AND AGE[1]

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In 1930 Anderson, Gayley, and Pratt (1), of this laboratory, published a paper on the chemical composition of bovine blood. In their work blood samples were taken at random from the dairy herd, the object being to determine normal values for many of the blood constituents. Perhaps the most interesting point brought out in their study was the wide variation in the glucose content of the blood of animals in the different age groups.

The work reported in this paper is a more detailed study of the effect of gestation and lactation on a few of the more important constituents of the blood which might be expected to vary under these conditions. There is also reported a detailed study of blood glucose values in calves' blood in an attempt to explain the high sugar values found as compared with the blood of older animals.

METHODS

Blood samples were taken between 6:00 and 7:00 A.M., usually before feeding. Two samples were drawn from the jugular vein, one in 20 mg. of potassium oxalate per 10 cc. of blood and the other without oxalate. The oxalated sample was used for the determination of non-protein nitrogen and glucose. From the sample without oxalate the serum was separated by means of a centrifuge within one hour after drawing and the serum used for the determination of calcium and phosphorus.

Non-protein nitrogen was determined by the method of Folin and Wu (7), and glucose by the method of Folin (6), on a laked blood filtrate. In most cases the glucose method was modified by using a standard containing 0.1 mg. of glucose per 2 cc. This was necessary because the glucose content of cow's blood is about one-half that of human blood.

Serum was analyzed for calcium by the method of Clark and Collip (4), and for phosphorus by the method of Youngburg (24).

In the studies on carbohydrate tolerance the method of Killian (13) was

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used. A technical grade of glucose was dissolved in water to make a 50 per cent solution and administered as a drench.

EXPERIMENTAL

1. *Influence of Gestation and Lactation on the Composition of Bovine Blood*

Since the complete analysis of blood requires much time, it was felt that the work might be lightened by a careful selection of determinations to be made. Many of the determinations made in a blood analysis are for nitrogenous constituents which are included in the non-protein nitrogen fraction. Therefore it was felt that non-protein nitrogen values would indicate whether or not any of the components of this fraction were varying. A knowledge of non-protein nitrogen values would also serve as an excellent measure of kidney function throughout the experimental period. Since previous work (1) had indicated that the glucose content of bovine blood varied considerably with age, it was felt that this determination should be included. Calcium and phosphorus determinations were included because during pregnancy and lactation extraordinary demands are placed on the reserves of these elements in the body.

In this study the blood of the experimental animals was analyzed at approximately weekly intervals from the time of breeding through the year of lactation. Since the time required to follow the same group of throughout the entire period of gestation and lactation was prohibitive, two groups of animals were used. Group I consisted of five heifers (two Holsteins, two Guernseys, and one Jersey) that had been bred for the first time and Group II consisted of five cows (four Holsteins and one Jersey) that had just dropped their first calf. The animals were selected from the purebred dairy herd of The Pennsylvania State College. They received the regular ration fed the dairy herd. All samples were analyzed in duplicate and the average of these results was used.

The data obtained in this study indicated that gestation and lactation had little effect on the blood constituents determined. Since the presentation of these data would require a table containing over 1,300 values, it appeared desirable to summarize the data in Table 1 and comment on a few of the results that perhaps are significant.

Non-protein nitrogen.—Non-protein nitrogen values ranged from 21.01 to 45.71 mg. per 100 cc. of blood, the average value being 32.82. These values do not differ significantly from those which one would expect to find for normal human blood. The average value during gestation is about 2.0 mg. less than the average value during lactation. The results agree with those of Anderson, Gayley and Pratt (1) and Hayden and Sholl (10).

Immediately after parturition non-protein nitrogen values were above

TABLE 1

Showing the composition of cow's blood from the time of breeding through one year of lactation

Number of animals	Constituents determined	Number of determinations	High	Low	Average
	From breeding to parturition				
				mg. per 100 cc.	
5	Non-protein nitrogen	164	45.71	21.01	31.83
	Glucose	171	68.00	60.70	52.51
	Calcium	166	12.90	7.70	10.73
	Phosphorus	161	7.54	4.16	5.64
	From parturition through lactation				
5	Non-protein nitrogen	168	45.58	21.24	33.82
	Glucose	171	52.33	37.80	51.73
	Calcium	166	13.20	9.00	11.08
	Phosphorus	164	7.49	3.38	5.23
	From breeding through lactation				
10	Non-protein nitrogen	332	45.71	21.01	32.82
	Glucose	342	68.00	37.80	52.12
	Calcium	332	13.20	7.70	10.91
	Phosphorus	325	7.54	3.38	5.44

average, the values of the five animals being 34.28, 39.09, 41.73, 43.33 and 44.11 mg.

During a period of severe cold weather, where the temperature remained below zero most of the time for about three weeks, non-protein nitrogen values were from 5 to 10 mg. higher than they were just previous and just after this period. Since the animals did not drink as much water as usual during the cold weather, this may explain the high results. Peters and Van Slyke (16) state that non-protein nitrogen values in human blood are increased with a low water intake.

Glucose.—The results for glucose gave an average of 52.12 mg. per 100 cc. of blood for 342 determinations. Values ranged from 31.8 to 71.4 mg. The low value is questionable, since for the previous week this animal gave a value of 46.6 mg. and on the following week 50.7 mg. At no other time throughout the experiment did the value for this animal go below 46.2 mg. The second lowest value for any animal was 37.8 mg. The average value of 52.12 mg. is in close agreement with that reported by Hodgson, Riddell and Hughes (12); Anderson, Gayley and Pratt (1); and Hayden and Sholl (10).

There was no significant difference in the glucose content of the blood of the pregnant non-lactating animals and of the lactating group. The former group gave an average of 52.51 mg. while the latter gave 51.73 mg. Considerable work has been reported on the effect of lactation on blood sugar values. Among those who have found low values for lactating animals may be mentioned Widmark and Carlens (23); Hewitt (11); Hodgson, Riddell and Hughes (12); Schlotthauer (19); Schwarz (20); Schwarz and Mezler-Andelberg (21). Moussu and Moussu (15) and Fish (5) report higher values for lactating cows than for dry cows. Scheunert and Pelchrzim (18); Awdejewa, Prowatorowa, Sawitsch, and Thal (3); and Hayden and Fish (9) found no difference in the blood sugar level between dry and lactating cows.

Calcium.—The values for calcium on blood serum were fairly constant throughout the experiment, most of the values falling between 9.0 and 11.5 mg. per 100 cc. As a result of 332 determinations, values were obtained ranging from 7.7 to 13.2 mg., the average value being 10.91 mg. per 100 cc. of serum. Pregnancy and lactation apparently had little effect on the calcium content of the blood, the average value for the former period being 10.73 mg. and for the latter 11.08 mg. Our results agree with the findings of Meigs, Blatherwick and Cary (14); Robinson and Huffman (17); and Haag (8).

Phosphorus.—The values for inorganic phosphorus in the serum remained fairly constant throughout pregnancy and lactation. As a result of 325 analyses, values were obtained ranging from 3.38 to 7.54 mg. per 100 cc. The average value was 5.44 mg. per 100 cc. During pregnancy the average value was 5.64 mg., while during lactation it was 5.23 mg. In previous work from this laboratory (1) it has been shown that serum phosphorus values decrease with age. Since in this experiment we were dealing with young animals bearing their first calf, it is quite possible that the slightly lower values for phosphorus during lactation are due to an age factor rather than to lactation. The above values agree closely with the results obtained by others (1, 14, 17).

2. *Blood Sugar Studies*

If one compares the composition of human blood with that of the cow, the most striking difference is found in the sugar content. Normal human blood contains in the neighborhood of 100 mg. of glucose per 100 cc. whereas cow's blood contains approximately 50 mg. per 100 cc. When the glucose value of human blood reaches 45 mg. per 100 cc. it is said to be at the convulsive level. Why a cow can be normal with the blood sugar at the convulsive level for humans is an interesting question. Since sheep (22) and goats (9) also have low blood sugar values it would appear that a low level of blood sugar is characteristic of ruminants. Dogs and horses (9) have blood sugar values approximating that of man, while chickens (9) and rats (2) have definitely higher values than man. From studies with the use of insulin in diabetes it is known that there is a definite relationship between the amount of insulin given to an individual and his blood sugar level. It would be an interesting study to determine the insulin content of the pancreases of different species and to observe any correlation between these findings and the normal blood sugar levels for these species.

Blood sugar and age.—A very interesting fact brought out in a previous study (1) of the blood sugar of bovines was that in young calves blood sugar values are twice as high as in mature cows. This has suggested a more detailed study of the influence of age on blood sugar values. For this study two groups of calves were selected; the first group consisting of three calves less than a week old and the second group of two calves five months of age. The two groups were selected in order to cover approximately the first year of life in the six months available for the work. Samples were taken usually at 8:30 A.M. after feeding and at weekly intervals covering the first 47 weeks of life. The results are shown graphically in Figure 1.

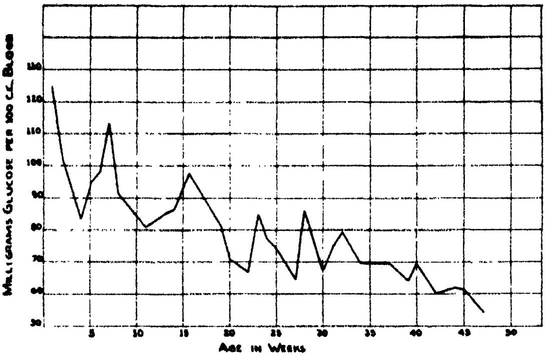

FIG. 1. Showing variation in blood sugar values of calves as influenced by age.

It may be observed from Figure 1 that for the first week of life the average blood sugar value was 125.0 mg. per 100 cc. of blood. By the end of the second week the average value had dropped to 102.5 mg. and by the end of the fourth week to 83.3 mg. For the next three weeks values increased and then they decreased. Considerable variation was observed until the thirtieth week when the curve became quite smooth to the end of the period when a value of 54.4 mg. was reached, which is about the value to be expected for a mature animal.

Diurnal variation in blood sugar.—The fluctuations in the first half of the curve shown in Figure 1 suggested a possible diurnal variation in blood sugar values. In order to study this an experiment was run on two calves over a period of 24 hours. Blood samples were taken frequently throughout this period. The results are shown graphically in Figure 2. The first samples were taken before feeding in the morning and the last samples before feeding the following morning. The blood sugar averaged 80.8 mg. per 100 cc. on the first morning and 80.5 mg. on the second. Samples taken one hour after the morning feeding showed an average glucose value of

129.4 mg. per 100 cc. This value gradually decreased until at 2:00 P.M. the value was 86.6 mg. The calves were fed again at 4:30 P.M. and blood samples were drawn again at 5:00 P.M. At this time the average glucose value was 114.2 mg. The reason that this value did not equal the level of 129.4 mg., reached one hour after feeding in the morning, is undoubtedly due to the fact that only 30 minutes elapsed from the time of feeding until the blood sample was drawn in this case. By 11:30 P.M. the glucose value had decreased to 96 mg.

From this experiment it is readily seen that there is a marked diurnal variation in the blood sugar values of calves which is associated with feeding. Since the calves were being fed mainly milk, the high blood sugar values may easily be accounted for by the high lactose content of the diet.

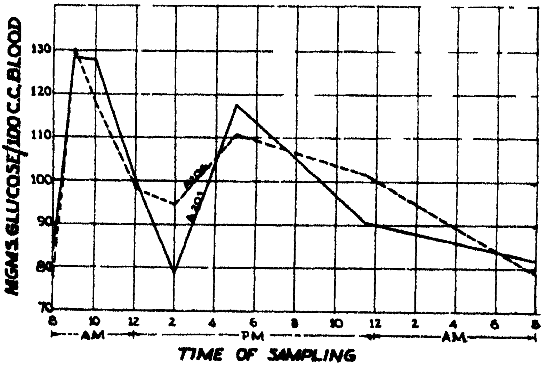

FIG. 2. Showing diurnal variation in blood sugar of calves less than one month of age.

Each calf received 13 pounds of milk a day which would contain about 0.6 pound of lactose. Thus at each feeding a calf received about 0.3 pound or 135 gm. of lactose. If in the case of a calf, milk passes directly into the abomasum and shortly into the intestine, there would be little delay in the digestion and absorption of lactose after milk is consumed.

When a carbohydrate tolerance test is given in human medicine the patient is given 1.75 gms. of glucose per kilogram of body weight. Since the average weight of the calves in this experiment was 87 pounds, the amount of sugar each received per feeding amounted to about 3.4 gms. per kilogram of body weight or about twice the amount given to a human in a sugar tolerance test. This would easily account for the rise in blood sugar after feeding. It is evident from this experiment that blood sugar values in calves must be interpreted on the basis of time of sampling in relation to time of feeding.

Sugar tolerance tests.—Although the feeding of large quantities of milk accounts in large measure for the high blood sugar values observed in the above experiment, it does not account for the fact that even before feeding, calves have higher blood sugar values than mature cows.

In human nutrition it is recognized that the digestive mechanism is not highly developed in an infant. An infant may thrive on its mother's milk but may fail to develop properly on a modified cow's milk. This may be due to the fact that the infant has the digestive enzymes capable of handling human milk while it may be lacking in enzymes capable of digesting the slightly modified molecules in cow's milk. It is quite possible that the same situation holds with regard to the endocrine system of a young animal. If the pancreas of a young animal produces a limited supply of insulin, this might easily account for the high blood sugar in young calves. It is possible that a calf may have diabetic tendencies.

In human medicine, when a person is suspected of having diabetes, it is customary to make a carbohydrate tolerance test in which 1.75 gms. of glucose per kilogram of body weight is fed to the individual and the influence of such treatment on the blood sugar level is determined by frequent blood analyses. In order to determine whether or not calves have less ability to utilize sugar than cows, the following carbohydrate tolerance tests were made. The results are shown graphically in Figure 3.

Eighteen animals ranging in age from less than one month to mature cows were divided into eight groups on the basis of age as indicated in Figure 3. Each animal was given a carbohydrate tolerance test by feeding 1.75 gms. of glucose per kilogram of body weight administered as a drench in a 50 per cent solution.

Immediately before feeding the sugar meal blood samples were taken and analyzed for glucose. After the sugar meal, blood samples were drawn at the end of one, two, three and four hours and likewise analyzed for glucose. The values are plotted in Figure 3 for each animal.

It is evident from Figure 3 that animals less than one month of age show a much less rapid utilization of glucose than the animals of the older groups. As age increases there is better and better utilization of glucose until in the bred heifer and lactating cow groups there is only a slight elevation of blood sugar values after the sugar meal. Animals A–202 and A–187 do not appear to fit into the general picture. In the case of A–187 a possible explanation may be that this calf had been allowed to nurse different cows until it was four months of age and thereby may have developed the ability of taking care of more sugar than the other calves. It should also be noted that both A–202 and A–187 started the carbohydrate tolerance test at much lower blood sugar levels than their mates. It is possible that this low initial blood sugar level indicates a glycogen depletion at the outset of the test and that the sugar fed was used immediately for the production of glycogen. However the odd responses of these two animals may be more

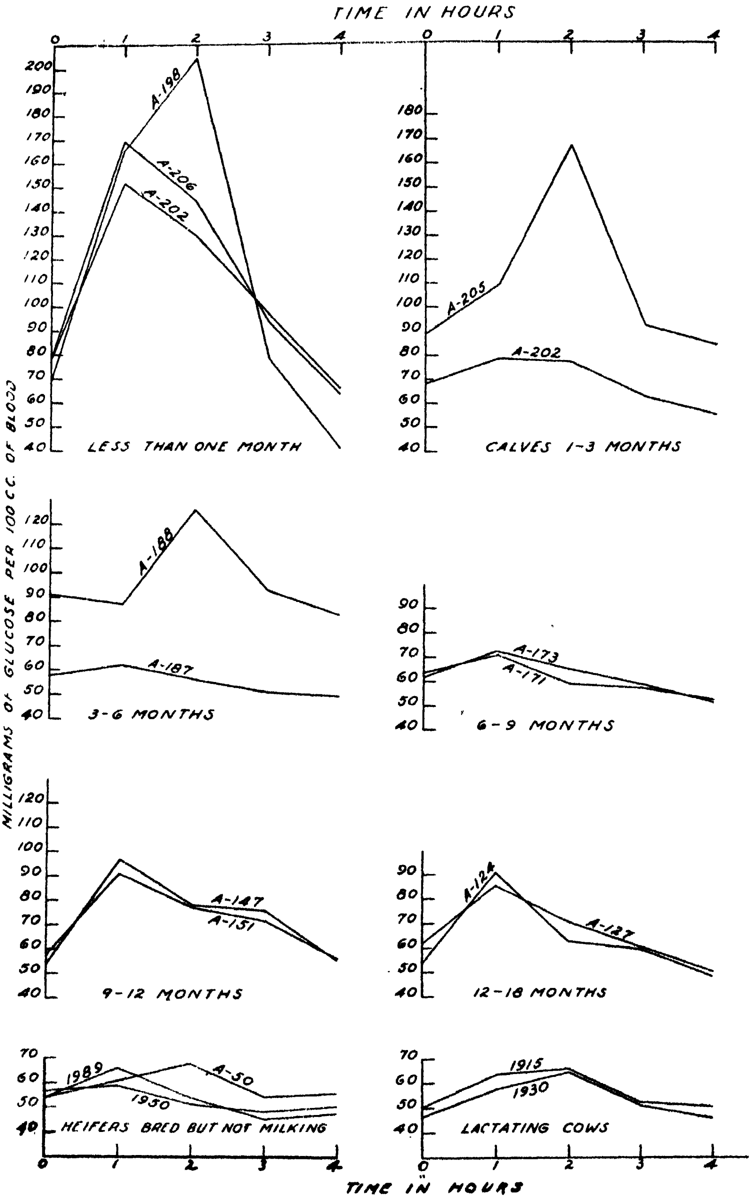

FIG. 3. Showing results of sugar tolerance tests on animals of various ages.

simply explained by assuming that they have developed an efficient glucose utilization mechanism at an early age.

From this experiment it is apparent that calves, especially very young calves, are less able to metabolize glucose than older animals. It would appear that the most logical explanation is that in very young calves the pancreas is not producing as much insulin as in older animals. It would be an interesting study to compare the insulin content of the pancreases of young calves and older cows. If it were found that the pancreases of older animals were richer in insulin than those of young animals this information might find a valuable application in the commercial manufacture of insulin.

SUMMARY

The blood of heifers has been analyzed at weekly intervals, from the time of breeding through one year of lactation, for non-protein nitrogen, glucose, calcium and phosphorus. There is little variation in these blood constituents as influenced by gestation and lactation. Average values for non-protein nitrogen were 32.82; for glucose 52.12; for calcium 10.91 and for phosphorus 5.44 mg. per 100 cc.

Blood glucose values are definitely related to age. Blood from calves less than one week of age contained 125 mg. of glucose per 100 cc. This value gradually decreased to 54.4 mg. per 100 cc. of blood at the end of a year.

There is a diurnal variation in the glucose content of calves' blood. Directly after feeding milk, blood sugar values increase sharply.

Carbohydrate tolerance tests on animals of various age groups indicate very little response to sugar feeding in the older groups but a decided response in young calves.

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CERTAIN FACTORS AFFECTING THE PHOSPHATASE TEST WHEN APPLIED TO ICE CREAM[1]

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INTRODUCTION

The value of the phosphatase test as a control measure in the pasteurization of market milk has been well established in this country and abroad. Both experimental results and field studies indicate that even minor deficiencies in the pasteurization of milk can be detected with this test (1, 2, 3, 4, 5). The question arises as to the adaptability of the phosphatase test in detecting similar deficiencies in the pasteurization of ice cream mix. Except for the data of Hahn and Tracy (6) no experimental results have been published pertaining to the application of the test to ice cream.

Lack of information on the subject and the need for a quick accurate test for detecting improperly pasteurized samples of ice cream mix prompted the initiation of a research project on this subject. Factors studied were: (1) ingredients of the mix, (2) vanilla flavoring used, (3) variations in the time and temperature of pasteurization, (4) contamination of pasteurized mix with raw products, (5) homogenizing and freezing processes, and (6) storage of the frozen ice cream.

PROCEDURE

Two phosphatase tests, the Gilcreas and Davis test (3) and the New York field or vest pocket test (4) were used throughout this investigation. In addition the Scharer laboratory test (4) was used in a part of the study. The latter test, however, was discontinued because of the difficulty in filtering the ice cream mix samples and because the filtrates obtained were cloudy in many cases. A recent report on the Scharer test indicates that certain modifications in the technic and reagents may have overcome these difficulties (7).

The recommended procedures for each of the phosphatase tests were followed unless otherwise stated (3, 4). Near the completion of this study refinements in reagents and technic recently recommended were introduced in connection with the New York field test (7). These changes consisted in using a buffer substrate solution containing magnesium ions and the addition of neutralized butyl alcohol to the sample for the purpose of concentrating any blue color into the alcohol layer. Results obtained with this newer technic will be especially mentioned.

The ice cream mix used was calculated to contain 12 per cent of fat, 10

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per cent of serum solids, 15 per cent of sugar, and 0.35 of one per cent of gelatine. Ingredients used in preparing the mixes included raw cream, raw skim or raw whole milk, condensed or skim milk powder, sugar, and gelatine.

Commercial pasteurization was carried out in either a 100-gallon glass-lined pasteurizer or a 10-gallon steam-jacketed copper kettle equipped with a mechanical agitator. The mix temperature was checked by means of an indicating thermometer accurate to ± 0.2° F. The holding time was checked by a stop-watch and an interval timer. All samples taken during or at the conclusion of the pasteurization treatment were held in stoppered test tubes immersed in an ice water bath until tested.

The usual commercial procedure was followed in homogenizing, freezing, and hardening of the mixes when the influence of these factors on the results of the phosphatase test were studied.

When laboratory pasteurization was used it consisted of immersing test tubes containing samples of the mix being treated in a water bath maintained at the proper temperature. The mix temperature was checked by inserting an indicating thermometer (accurate to ± 0.2° F.) into a check tube of the mix. The holding time was controlled by the same timing devices mentioned in connection with commercial pasteurization.

EXPERIMENTAL RESULTS

Effect of various ingredients in the ice cream mix.—Sugar was found to exert a protective action against the inactivation of phosphatase when ice cream mixes were pasteurized commercially at 142, 143.5, and 145° F., respectively (Table 1). When mixes containing sugar were pasteurized at these temperatures for 30 minutes positive phosphatase results were obtained in all cases but when portions of these same mixes were pasteurized before the sugar was added, negative results were obtained.

TABLE 1

Influence of sugar on the inactivation of phosphatase in ice cream mixes as measured by two phosphatase tests when pasteurized at 142, 143.5, and 145° F., respectively, for 30 minutes. (Commercial pasteurization)

Pasteurization temperature (deg. F.)	Description of sample	Phosphatase test results (av. of 2 trials)	
		Gilcreas and Davis test*	New York field test
142	Complete mix	>0.150	+++
142	Mix without sugar	0.020	---
142	Sugar added after pasteurization	0.020	---
143½	Complete mix	>0.150	++
143½	Mix without sugar	0.020	--
145	Complete mix	0.120	++
145	Mix without sugar	0.015	--
145	Sugar added after pasteurization	0.015	--

* Results expressed as mg. of phenol per 0.5 ml. of sample. Values in excess of 0.037 mg. of phenol are considered definitely positive.

The addition of sugar to the mixes after pasteurization, at 142 and 145° F. for 30 minutes, had no effect on the results of the phosphatase tests (Table 1). It is evident that the positive phosphatase reactions observed when mixes containing sugar were pasteurized at these temperatures were not due to the sugar itself but to the protective action of the sugar during heat treatment. A similar relationship between sugar and the destruction of bacterial cells was observed by Fay (8).

The data obtained in this study show that pasteurization of ice cream mixes containing sugar at 142–145° F. for 30 minutes was not adequate to inactivate the phosphatase present. Ice cream mixes thus pasteurized would all be adjudged as under-pasteurized on the basis of the phosphatase test. Furthermore, it would not be possible to detect differences in the pasteurization temperatures used within the limits 142–145° F. on the basis of the phosphatase test results (Table 1). These data as well as those obtained by Fay (8) indicate the necessity for using higher pasteurization temperatures with ice cream mixes than are ordinarily employed in the pasteurization of market milk.

Although sugar exerted a protective effect against the inactivation of phosphatase in mixes pasteurized at 142–145° F., no such effect was apparent when the pasteurization temperature was 150° F. (Table 2). Laboratory pasteurization at 150° F. for 30 minutes of various combinations of ice cream mix ingredients yielded negative phosphatase results in each of a series of three trials, irrespective of the presence of sugar (Table 2).

TABLE 2

Influence of ice cream mix ingredients on the inactivation of phosphatase as measured by three phosphatase tests when pasteurized at 150° F. for 30 minutes.
(Laboratory pasteurization)

Ingredients used	Phosphatase test results (av. of 3 trials)		
	Gilcreas and Davis test*	Scharer laboratory test	New York field test
Milk and cream, raw	0.023	–†	–†
Milk, cream, and sugar	0.020	–	–
Milk, cream, and gelatine	0.020	–	–
Milk, cream, and condensed	0.020	–	–
Milk, cream, and powder	0.020	–	–
Complete mix	0.022	–	–

* Results expressed as mg. of phenol per 0.5 ml. sample. Values in excess of 0.037 are considered positive.

† Negative result.

Effect of vanilla flavors.—Ten different vanilla flavors (five pure bean, three reinforced, and two imitation) were used alone and in combination with ice cream mixes, and the samples tested with one or more phosphatase tests.

The pure bean vanillas, except for the concentrate, had no effect on results of the phosphatase test irrespective of the amounts used (Table 3).

When the pure bean vanilla concentrate was added directly to the buffer solution, a positive reaction to the New York field test was obtained. Also, a significant increase in the phenol value of the mix, as measured by the Gilcreas and Davis test, was obtained when double the recommended amount of flavor was added to the mix. When the pure bean vanilla concentrate was used according to the manufacturer's recommendations, however, both of the phosphatase tests yielded negative results.

Each of the reinforced or imitation vanilla flavors when added directly to the buffer solution gave a positive reaction by the New York field test. This observation is in harmony with the findings of Hahn and Tracy (6), who found that vanillin and coumarin resulted in positive reactions with the Scharer laboratory test. When used as recommended with ice cream mixes, only one of three samples (a reinforced vanilla) gave a positive reaction. This sample was positive only to the Gilcreas and Davis test.

When double the standard amount of reinforced or imitation vanillas were used, four of the five caused positive results with both phosphatase tests.

TABLE 3

Influence of certain vanilla flavors on the results of the phosphatase test.
(Average of two trials)

Sample number	Type of vanilla flavor used	Quantity of flavor used				
		1 drop to 5 cc. of buffer solution	Recommended amount for ice cream		Double the recommended amount for ice cream	
		Field test*	G & D test†	Field test	G & D test	Field test
1	Pure, concentrate	+	0.015	–	0.030	–
2	Pure	–	0.015	–	0.020	–
3	Pure	–	0.015	–	0.020	–
4	Pure	–	0.015	–	0.020	–
5	Pure	–	0.015	–	0.020	–
6	Reinforced	+	0.015	–	0.020	–
7	Reinforced	+	0.035	–	0.120	+
8	Reinforced	+	0.015	–	0.040	+
9	Imitation, concentrate	+	0.015	–	0.070	+
10	Imitation	+	0.015	–	0.060	+
11	Check (no flavor used)	–	0.015	–	0.020	–

* New York field test.

† Gilcreas and Davis test. Results expressed as mg. of phenol per 0.5 ml. sample. Values in excess of 0.037 are considered positive.

The data indicate that certain vanilla flavors, if used in excess, may cause a significant increase in the phenol value of an ice cream sample. It is important, therefore, to take into consideration the possibility of such interference when interpreting the results of phosphatase determinations on samples of vanilla ice cream.

Effect of variation in pasteurization temperature and time of holding.—

The results of six commercial and four laboratory pasteurization trials at 150° F. indicate that with but two exceptions the samples were negative to the Gilcreas and Davis phosphatase test at the end of a twenty-minute holding period (Table 4). In no case was a positive test obtained after a holding period of 25 minutes or more at 150° F.

The New York field test yielded negative results in all cases after a holding period of twenty minutes or more had elapsed, and with but two exceptions the results were negative after a 15-minute period (Table 4).

Mixes pasteurized at 160° F. were negative to the phosphatase tests after a five-minute holding period.

Variations noted in the time required for a mix to become negative to

TABLE 4

Effect of variations in pasteurization temperature and holding time on the inactivation of phosphatase in ice cream mixes

Holding time in minutes	Phosphatase test results											
	Gilcreas and Davis test						New York field test					
	Commercial pasteurization at 150° F.											
	Trial number						Trial number					
	1	2	3	4	5	6	1	2	3	4	5	6
0	++	++	++	++	++	++	++	++	++	++	++	++
5	++	++	++	+	++	++	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	–	–	+
15	–	+	+	–	–	+	–	–	–	–	–	–
20	–	–	–	–	–	+	–	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–	–	–	–
30	–	–	–	–	–	–	–	–	–	–	–	–
	Laboratory pasteurization at 150° F.											
	1	2	3	4	1	2	3	4				
0	++	++	++	++	++	++	++	++				
10	+	+	+	+	+	+	+	+				
15	+	–	+	–	+	–	+	–				
20	–	–	+	–	–	–	–	–				
25	–	–	–	–	–	–	–	–				
30	–	–	–	–	–	–	–	–				
	Commercial pasteurization at 160° F.											
	1	2	3	4	5	6	1	2	3	4	5	6
0	–	+	+	+	+	+	–	+	+	+	+	–
5	–	–	–	–	–	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–	–	–	–	–
30	–	–	–	–	–	–	–	–	–	–	–	–
	Laboratory pasteurization at 160° F.											
	1	2	3	4	5	6	1	2	3	4	5	6
0	++	++	++	++	++	++	++	++	++	++	++	++
5	–	–	–	–	–	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–	–	–	–	–
30	–	–	–	–	–	–	–	–	–	–	–	–

the phosphatase test when held at a specific temperature may be attributed largely to variations in the time required to heat the batch from 140° F. to the final pasteurization temperature. It is within this temperature zone that some phosphatase destruction occurs. Hence, the longer the time required to heat the mix through this temperature zone the lower will be the initial phosphatase content of the sample at the start of the holding period. This evidently explains why in several instances mixes pasteurized under laboratory conditions, where the heating takes place rapidly, remained positive to the phosphatase tests longer than did the mixes pasteurized with commercial equipment.

When ice cream mixes are pasteurized at 150° F. it is evident that the holding period might be shortened as much as 15 minutes before it would be detected with the Gilcreas and Davis phosphatase test. Similarly a reduction in the holding period of as much as 20 minutes could occur before it would be detected with the New York field test. In evaluating phosphatase test results on ice cream mixes it must be kept in mind, therefore, that a negative test does not mean that the mix has necessarily been held at 150° F. for a full thirty-minute period.

Similarly mixes pasteurized at 160° F. and held for five minutes would be adjudged efficiently pasteurized on the basis of the phosphatase test.

Detection of recontamination with a mixture of raw milk and cream.— In a series of six trials, the addition of 0.2 per cent or more of a mixture of raw milk and cream to a sample of pasteurized mix was detected in all cases with the Gilcreas and Davis test (Table 5).

With the New York field test, without the recently recommended refine-

TABLE 5

Sensitivity of phosphatase tests to the addition of raw milk and cream to pasteurized ice cream mixes

Sample number	Raw product added (per cent)	Phosphatase test results											
		Gilcreas and Davis						Field test (a)			Field test (b)		
		Trial No.						Trial No.			Trial No.		
		1	2	3	4	5	6	1	2	3	1	2	3
1	0.00	–	–	–	–	–	–	–	–	–	–	–	–
2	0.10	No samples			+	–	–	–	–	–	No samples		
3	0.20	+	+	+	+	+	+	–	–	–	–	–	–
4	0.30	+	+	+	+	+	+	–	–	–	+*	+*	+*
5	0.40	+	+	+	+	+	+	–	–	–	+*	+*	+*
6	0.50	+	+	+	+	+	+	+	+	–	+	+	+
7	0.75	+	+	+	+	+	+	+	+	+	+	+	+
8	1.00	+	+	+	+	+	+	+	+	+	+	+	+

* Samples gave a positive reaction only when butyl alcohol was used.

Field Test (a) = Original di sodium phenyl phosphate buffer substrate no butyl alcohol

Field Test (b) = New buffer substrate solution containing magnesium ions plus the use of butyl alcohol.

ments the presence of less than 0.75 per cent of added raw milk and cream was not detected in all cases. The use of the newer buffer substrate solution containing magnesium ions together with butyl alcohol in evaluating the final results increased the sensitivity of the New York field test to a point where 0.3 of one per cent of added raw milk and cream were detected without exception.

Effect of homogenization and freezing.—The phenol value of ice cream mixes taken before and after homogenization showed no significant changes, irrespective of whether the sample was negative or positive before treatment (Table 6). Similar results were obtained with samples of ice cream mix taken before and after freezing

TABLE 6

Effect of homogenization on the phenol value of ice cream mixes as measured with the Gilcreas and Davis phosphatase test

Pasteurization treatment of mix	Number of trials	Phosphatase test results	
		Before homogenization (Ave. mg. phenol per 0.5 ml. sample)	After homogenization (2500 pounds' pressure) (Ave. mg. phenol per 0.5 ml. sample)
145° F. 30 min	5	.150	.150
150° F. 30 min	6	0.018	0.018
160° F. 30 min.	6	0.012	0.014*

* Sample taken at the completion of the freezing process, all other samples taken immediately before or after homogenization.

Effect of time and temperature of storage.—No marked change was observed in the phenol values of ice cream samples stored for two weeks in either an ice cream hardening room at approximately (- 10° F.) or in a mechanically refrigerated cabinet at approximately (+ 10° F.) (Table 7). Longer storage periods than two weeks, however, have yielded somewhat variable results. Positive tests were observed with three additional sets of ice cream samples after a three weeks' storage period in both the cabinet and hardening room. All of these samples, however, were negative at the end of two weeks storage. In another trial, one of three sets of duplicate samples remained negative throughout a storage period of one month. Of the two other sets of samples one was positive in both the cabinet and hardening room, while only the sample maintained in the cabinet was positive in the remaining set after a month's storage period. No explanation for the factors responsible for these changes in the phenol values during storage can be given.

TABLE 7

Influence of time and temperature of storage on the phenol value of ice cream samples as measured with the Gilcreas and Davis phosphatase test

Pasteurization treatment of mix	Number of trials	Age of samples in weeks					
		Fresh		1		2	
		Place and temperature of storage					
		Cabinet (+10° F.)	Hardening room (−10° F.)	Cabinet (+10° F.)	Hardening room (−10° F.)	Cabinet (+10° F.)	Hardening room (−10° F.)
		Ave. phosphatase test results*					
145° F. 30 min.	5	>0.150	>0.150	>0.150	>0.150	>0.150	>0.150
150° F 30 min.	8	0.023	0.024	0.027	0.027	0.027	0.027
160° F. 30 min.	5	0.017	0.017	0.023	0.021	0.023	0.024

* Values expressed as mg. of phenol per 0.5 ml. sample. Values in excess of 0.037 mg. of phenol are considered positive.

The results indicate that up to two weeks there is apparently little or no change in the phenol values of ice cream and that samples may be dependably tested any time during that period.

DISCUSSION

Results obtained in this study support the conclusions of Hahn and Tracy that the phosphatase test, with certain limitations, may be used as an index to satisfactory pasteurization of ice cream or ice cream mixes. Since one of these limitations is the possible effect of imitation or reinforced vanilla flavors on the results of the tests, it would be desirable, whenever possible, to apply the test to the unflavored mix. If this is not possible, the precaution should be taken to run a suitable control test on all positive samples to avoid misinterpretation of the results.

A negative phosphatase test on a sample of mix does not necessarily mean that the sample has been pasteurized at 150° F. for 30 minutes, which is the legal requirement for the pasteurization of ice cream mixes now in effect on many markets. Negative phosphatase results were obtained in this study after a holding period of 15 to 25 minutes at this temperature. Similarly a holding period of 5 minutes proved sufficient to inactivate phosphatase when a pasteurization temperature of 160° F. was used. Hence, it would be difficult to enforce a legal standard requiring a pasteurization temperature of 150 or 160° F. for 30 minutes with the phosphatase tests used in this study. On the other hand, results obtained in this study show that the phosphatase test is capable of detecting mixes which have not been heated in excess of 145° F for 30 minutes.

The fact that the holding period with pasteurization temperatures of 150 or 160° F. could be materially shortened before it would be detected with either of the phosphatase tests used in this study raises the question as to just what constitutes efficient pasteurization of ice cream mixes. Can a mix which is negative to the phosphatase test be considered efficiently pasteurized even though it may have been held for only 15 minutes at 150° F. or 5 minutes at 160° F.? Obviously the true answer to the question must be determined on the basis of the bacteriological flora surviving such treatments. If such studies demonstrate that the mix is pasteurized satisfactorily from a bacteriological viewpoint before it becomes negative to the phosphatase test, then the value of the test as a true index to the satisfactory pasteurization of ice cream mixes will be greatly increased. Until the results of such a study are known, however, it would be unsafe to assume that negative phosphatase tests are necessarily indicative of proper pasteurization. On the other hand, positive tests would always be indicative of inefficient pasteurization.

Both the New York field and Gilcreas and Davis tests were found to be extremely sensitive to the addition of raw products to a sample of properly pasteurized mix. The tests should prove extremely useful in detecting the indiscriminate dilution of properly pasteurized mix with raw milk or cream.

Although not perfect, the phosphatase tests used in this study offer greater possibilities for insuring adequate pasteurization of ice cream than has been available up to the present time.

SUMMARY AND CONCLUSIONS

A study has been completed to determine whether or not the phosphatase test can be satisfactorily used to determine the pasteurization efficiency of ice cream. Factors studied were: (1) ingredients, (2) vanilla flavors, (3) variations in the time and temperature of treatment, (4) contamination of pasteurized mix with raw products, (5) homogenization and freezing, and (6) storage of the frozen ice cream.

The more important results of this study may be summarized as follows:

1. Sugar in the mix was found to protect phosphatase against inactivation by heat when pasteurization temperatures of 142, 143.5 and 145° F. for 30 minutes respectively were used. Portions of these same mixes, but pasteurized without sugar, gave negative phosphatase reactions at these same temperatures.
2. When the pasteurization temperature was increased to 150° F. for 30 minutes negative phosphatase results were observed in all cases irrespective of the presence of sugar in the mix.
3. Certain vanilla flavors if used in excess were found to cause a significant increase in the phenol value of an ice cream mix.
4. The data show that the usual 30 minute holding period may be

materially shortened before it would be detected by either the Gilcreas and Davis, or New York field phosphatase tests when ice cream mixes are pasteurized at 150° F. or higher.

5. It was possible with the Gilcreas and Davis test to detect the admixture of as little as 0.2 per cent added raw milk and cream to a sample of pasteurized mix.

Seventy-five hundredths of one per cent added raw milk and cream could be detected with the original New York field test and with a modified technic it was possible to detect the presence of 0.3 per cent added raw products.

6. No differences were observed in the results of the phosphatase tests as a result of homogenization or freezing.

7. Samples of ice cream stored for two weeks in an ice cream cabinet (at approximately + 10° F.) and in an ice cream hardening room (at approximately − 10° F.) showed no significant change in their phenol values.

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ASCORBIC ACID AND OXIDIZED FLAVOR IN MILK. I. DISTRIBUTION OF ASCORBIC ACID AND OCCURRENCE OF OXIDIZED FLAVOR IN COMMERCIAL GRADE A RAW, IN PASTEURIZED IRRADIATED, AND IN PASTEURIZED MILK THROUGHOUT THE YEAR[1]

G. M. TROUT AND ERLAND C. GJESSING

Recent developments in the chemical aspects of vitamin C, in its isolation, and in the exact chemical methods for its determination have made possible rapid and accurate methods for determining the amount of vitamin C as ascorbic acid in a great variety of foods. The determination of ascorbic acid in milk by chemical methods has recently been made possible.

Sharp (5), using the rapid 2–6 dichlorophenolindophenol titration method (6) for the determination of reduced ascorbic acid, showed that the ascorbic acid of milk holder pasteurized in glass was as stable as that in the raw product similarly handled to prevent metal contamination. He also demonstrated (4) the high correlation which existed between the presence of the oxidized flavor and the almost total lack of ascorbic acid in milk.

Whitnah *et al.* (8) observed an inverse relationship between the naturally occurring vitamin C in breed milk and the frequency of the development of the oxidized flavor. However, they found no relationship between the amount of vitamin C and the development of oxidized flavor in milk from individual cows.

Rasmussen *et al.* (2) mentioned the apparent relation between the "cardboard" flavor and a low ascorbic acid content of milk. They observed that samples of milk which developed cardboard flavor on standing invariably decreased in ascorbic acid coincident with the formation of the off-flavor.

Inasmuch as this oxidized flavor is usually a seasonal problem, disappearing in the late spring and recurring in the fall and winter, an understanding of the rôle of ascorbic acid in commercial milk throughout the year seemed desirable. Consequently, the major portion of the data reported herein was obtained by routine analysis from daily, bi-weekly, or tri-weekly samples of commercially processed milk throughout the year.

EXPERIMENTAL

Samples of grade A raw, pasteurized irradiated, and regular pasteurized milk taken daily, bi-weekly, or tri-weekly throughout the year were titrated for ascorbic acid daily for four days. The milk was titrated with 2–6 dichlorophenolindophenol using the rapid method of Sharp (6).

The grade A raw milk, which averaged approximately 4.5 per cent fat,

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was produced at the College Dairy Barn. Here the milk was machine drawn into aluminum containers from which it was poured over a surface cooler; stored in 10-gallon cans; the noon, night, and morning's milk mixed and bottled daily in a rotary bottler.

The irradiated milk was essentially the same as the grade A raw milk with the exception that it was irradiated in a CP irradiator and then pasteurized at 142°–143° F. for 30 minutes.

The regular pasteurized mixed milk, testing 3.7 per cent fat, was obtained from approximately 30 producers delivering milk to the College Creamery. The milk was holder pasteurized in glass-lined or in Allegheny metal pasteurizers.

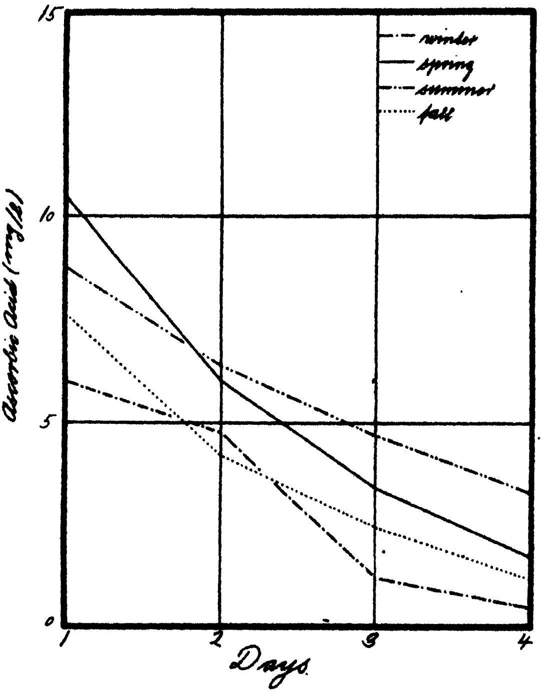

FIG. 1. Seasonal variation of ascorbic acid in grade A raw milk after different periods of storage.

Pint samples of the stored bottled milk were secured at random at time of loading and delivery and portions from each bottle were titrated for ascorbic acid each day for four successive days. The milk obtained for titration, therefore, was night's and morning's milk mixed, processed, and stored for distribution the following day. Consequently, the age of the milk at time of the first day's titration was from 24 to 34 hours.

TABLE 1

Percentage distribution of samples of grade A raw milk according to ascorbic acid content by seasons

Ascorbic acid	Grade A raw milk held four days at 40° F.: percentage distribution of															
	Winter samples				Spring samples				Summer samples				Fall samples			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
Mg. per liter	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0– 0.99		7.1	61.0	75.1		11.1	18.5	36.7			3.3	12.9	2.9	3.2	22.3	54.6
1.00– 1.99	3.3	21.4	16.7	18.8	2.3	11.1	22.2	33.3			13.3	19.4	5.9	19.4	33.3	27.3
2.00– 2.99		7.2	5.6			8.3	3.7	10.0	4.4	15.8	16.6	25.8	11.7	22.5	22.2	9.1
3.00– 3.99	3.3	14.3	5.6	6.3	4.7	2.8	11.1	6.7	8.9	5.3	13.3	6.4		16.1	5.6	9.1
4.00– 4.99	13.3	7.2	5.6		7.0	13.9	14.8	10.0	8.9	13.2	6.7	9.7	5.9	12.9	5.6	
5.00– 5.99	6.7	10.7	5.6		2.3	5.6	14.8		2.2	15.8	10.0	12.9	5.9	3.2		
6.00– 6.99	6.7	17.9			2.3	5.6	7.4		11.1	15.8	20.0	3.2	5.9	3.2		
7.00– 7.99	13.3				4.7	8.3	3.7	3.3	6.8	2.6	3.3	9.7	8.8	6.6	5.6	
8.00– 8.99	13.3	7.2			7.0	5.6	3.7		4.4	13.2	6.7		20.6		5.6	
9.00– 9.99	13.3	3.6			9.3	11.1			11.1	5.3	3.3		2.9	3.2		
10.00–10.99	6.7	3.6			9.3	8.3			6.8	2.6	3.3		2.9	3.2		
11.00–11.99	3.3				14.0	2.8			11.1	7.9			8.8	3.2		
12.00–12.99	3.3				2.3	5.6			2.2	2.6			5.9	3.2		
13.00–13.99	10.0				11.6				2.2				5.9			
14.00–14.99	3.3				11.6				13.3				2.9			
15.00–15.99					7.0				4.4							
16.00–16.99					2.3				2.2				2.9			
17.00–17.99					2.3											
No. of samples	30	28	18	15	43	36	27	30	45	38	30	31	34	31	18	11

RESULTS

The percentage distributions of the samples of grade A raw, pasteurized irradiated, and regular pasteurized milk, according to ascorbic acid content, by seasons, are given in Tables 1, 2, and 3. The average seasonal values, presented in Table 4, are shown graphically in Figures 1, 2, and 3. The percentage decreases in ascorbic acid and the incidence of oxidized flavor during the seasons are given in Table 4.

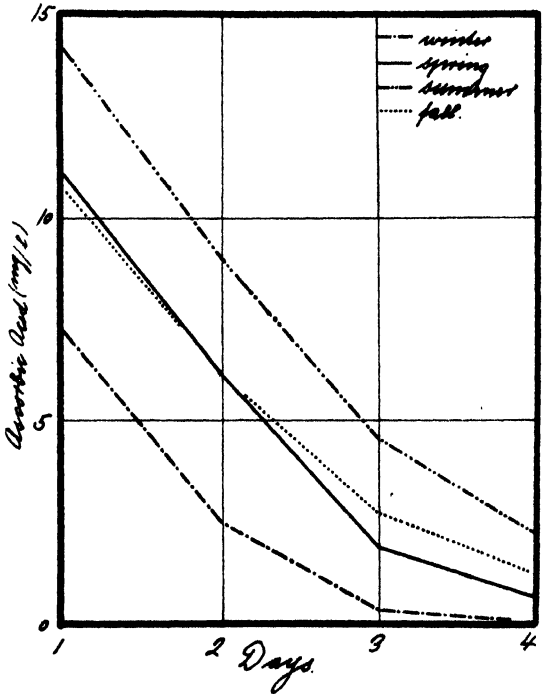

FIG. 2. Seasonal values of ascorbic acid in irradiated milk after different periods of storage.

Grade A raw milk.—The percentage distribution of samples of grade A raw milk by ascorbic acid content and by season is shown in Table 1. A comparison of the distribution values for the third and fourth day's titration for winter, spring, summer and fall respectively shows the relatively high percentage of the samples having little or no ascorbic acid during the fall and winter months and the slightly greater amounts of ascorbic acid present in milk held for similar periods during the spring and summer months. However, as shown in Table 4, on the first day of titration, 6.0, 10.5, 8.8, and 7.7 mg. per liter of ascorbic acid were present in the grade A milk during the winter, spring, summer and fall seasons respectively. The average ascorbic acid value for the first day throughout the year was 8.3

TABLE 2

Percentage distribution of samples of pasteurized irradiated milk according to ascorbic acid content by seasons

Ascorbic acid	Pasteurized irradiated milk held four days at 40° F.; percentage distribution of															
	Winter samples				Spring samples				Summer samples				Fall samples			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
Mg. per liter	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0– 0.99		18.0	46.7	100.0		4.8	11.4	71.5			2.9	14.3		10.7	25.0	52.6
1.00– 1.99	2.3	25.6	43.3				14.3	14.3		2.4		25.7			10.0	26.3
2.00– 2.99	4.5	10.3	10.0			4.8	31.4	7.1			20.0	37.1		3.6	15.0	10.5
3.00– 3.99		28.2				7.1	17.1	4.6		2.4	20.0	14.3		3.6	25.0	10.5
4.00– 4.99	4.5	17.9				9.5	11.4	2.3			14.3	5.7		10.7	15.0	
5.00– 5.99	18.2					14.3	5.7			2.4	17.1	2.8	2.9	10.7	5.0	
6.00– 6.99	9.1					23.8	5.7			9.6	11.4			28.6		
7.00– 7.99	25.0					19.0				14.4	5.7		2.9	14.3		
8.00– 8.99	18.2				2.0	9.5	2.9			16.8	2.9		11.7	3.6	5.0	
9.00– 9.99	11.4				2.0	2.4				16.8	2.9		14.7	3.6		
10.00–10.99	6.8				16.3	4.8				16.8	2.9		23.5	3.6		
11.00–11.99					18.4				8.7	4.8			11.7	7.2		
12.00–12.99					28.6				16.7	4.8			17.6			
13.00–13.99					18.4				21.7	2.4			2.8			
14.00–14.99					12.2				15.2				5.9			
15.00–15.99					2.0				19.4	2.4			5.9			
16.00–16.99									13.0							
17.00–17.99									4.3							
No. of samples	44	39	30	33	49	42	35	43	46	41	35	35	34	28	20	19

TABLE 3

Percentage distribution of samples of regular pasteurized milk according to ascorbic acid content by seasons

Ascorbic acid	Pasteurized milk held four days at 40° F.; percentage distribution of															
	Winter samples				Spring samples				Summer samples				Fall samples			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
Mg. per liter	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0– 0.99		2.6	14.2	63.6			9.4	15.2				3.0				29.4
1.00– 1.99		5.1	14.3	22.7		7.0	9.4	36.4			3.1	6.1			5.3	11.8
2.00– 2.99		10.3	25.0	4.5			6.3	12.1		2.6	6.2	33.3		3.3	15.8	11.8
3.00– 3.99	2.3	7.7	17.9	4.5		4.6	18.8	30.4			9.4	21.2			10.5	5.9
4.00– 4.99		10.3	7.2	4.5		2.3	9.4				18.7	24.2		10.0	10.5	11.8
5.00– 5.99	4.5	12.8	7.2			4.6	18.8			2.6	15.6	9.1		3.3	5.3	5.9
6.00– 6.99	9.1	15.4	10.7			9.3	15.6	3.0		2.6	12.5	3.0	2.6	10.0		
7.00– 7.99	9.1	12.8	3.6		1.9	23.2	9.4	3.0		7.7	18.7		5.3	3.3	21.1	11.8
8.00– 8.99	2.3	17.9				25.6				15.4	12.5			10.0		11.8
9.00– 9.99	18.2	5.1			3.9	11.6	3.1			28.2			15.8	16.7	5.3	
10.00–10.99	15.9				3.9	7.0				20.5	3.1		2.6	3.3	21.1	
11.00–11.99	13.6				15.7	2.3			6.8	12.8			5.3	6.6	5.3	
12.00–12.99	20.5				31.4				9.1	5.1			7.9	26.6		
13.00–13.99	4.5				15.7				20.5	2.6			18.4	6.7		
14.00–14.99					11.8	2.3			43.2				10.5			
15.00–15.99					11.8				13.6				18.4			
16.00–16.99					1.9				4.5				7.9			
17.00–17.99					1.9				2.3				5.3			
No. of samples	44	39	28	22	51	43	32	33	45	39	32	33	38	30	19	17

mg. per liter which was a lower average than that found in either the irradiated milk or in the regular pasteurized milk, under similar storage conditions. Graphical presentation of these data is shown in Figure 1. Here it will be observed that the amount of ascorbic acid in grade A raw milk was consistently higher in spring and summer than in the fall and winter. The percentage decrease of ascorbic acid upon storage was greater in the winter than in the spring, summer, or fall. The percentage decreases of ascorbic acid, after three days' storage during these seasons were respectively 78.3, 57.6, 46.5 and 67.5 (Table 4).

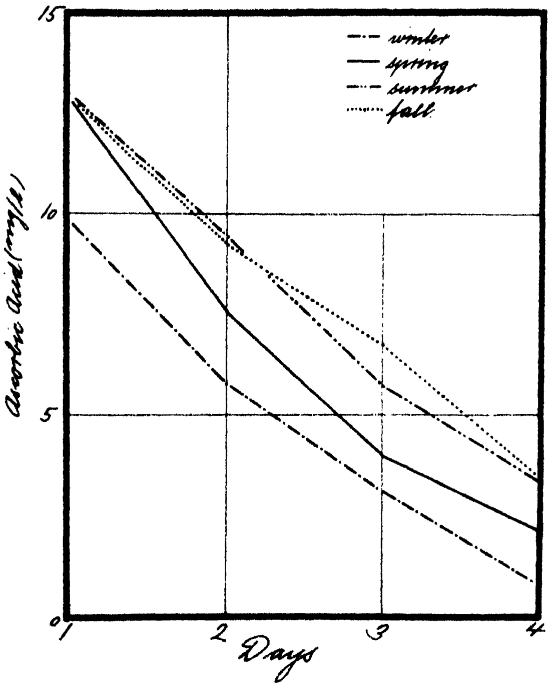

FIG. 3. Seasonal values of ascorbic acid in pasteurized milk after different periods of storage.

From late fall and throughout the winter, the grade A raw milk was occasionally criticized for its oxidized flavor. Organoleptic examinations revealed during this period slight to distinct oxidized flavor developments when the milk was held from 48 to 72 hours, but no oxidized flavors were noted in this milk during spring and summer. The grade A raw milk was produced free from copper contamination, and under sanitary conditions which resulted in average bacterial counts below 10,000 per cc.

Pasteurized irradiated milk.—The percentage distribution of the samples of pasteurized irradiated milk by ascorbic acid content and by season is shown in Table 2. During the winter months, 90 per cent of the samples showed less than 2.0 mg. per liter of ascorbic acid remaining on the third day of titration. On the fourth day the ascorbic acid had disappeared entirely from all the samples. As shown in Table 4, little difference was noted in the amount of ascorbic acid present in the spring and fall; however, at the third and fourth titration both were notably low. The ascorbic acid content of summer milk was low also on the third and fourth days of titration, but was higher than that observed at the other seasons.

TABLE 4

Average seasonal values of ascorbic acid in grade A raw, in pasteurized irradiated, and in pasteurized milk with the oxidized flavor noted

Season	Ascorbic acid mg. per liter at				Per cent decrease in ascorbic acid from 1st to 3rd day at which time samples were examined for flavor	Oxidized flavor development usually noted after 3 days*
	1st day	2nd day	3rd day	4th day		
			Grade A milk			
Winter	6.0	4.8	1.3	0.6	78.3	+ +
Spring	10.5	6.0	3.4	1.8	57.6	–
Summer	8.8	6.4	4.7	3.3	46.5	-
Fall	7.7	4.2	2.5	1.2	67.5	? +
Average	8.3	5.4	3.0	1.7		
			Pasteurized irradiated milk			
Winter	7.2	2.5	0.4	0.0	94.4	+ + +
Spring	11.1	6.1	2.0	0.7	81.9	+
Summer	14.2	9.1	4.7	2.2	66.9	–
Fall	10.9	6.1	2.8	1.2	74.3	+
Average	10.9	6.0	2.5	1.0		
			Pasteurized milk			
Winter	9.9	5.8	3.1	0.8	68.7	–
Spring	12.9	7.6	4.0	2.2	68.9	–
Summer	13.0	9.5	5.8	3.3	55.4	–
Fall	13.0	9.4	6.8	3.4	47.7	–
Average	12.2	8.1	4.9	2.4		

* Key: – no oxidized flavor
? doubtful oxidized flavor
+ slight oxidized flavor
+ + distinct oxidized flavor
+ + + very strong oxidized flavor

The first day values for ascorbic acid in irradiated milk throughout the year were 7.2, 11.1, 14.2, and 10.9 mg. per liter for the winter, spring, summer and fall seasons respectively, these values being higher than those found in grade A milk under similar storage (Table 4). The values graphically presented in Figure 2 show that the average ascorbic acid content per day of storage in summer milk was approximately as much above that of spring and fall milk as that in winter milk was below the spring and fall values.

Again, the percentage decrease of ascorbic acid in stored winter milk was higher than that observed during the other seasons. The percentage seasonal decreases of ascorbic acid from the first to the third day of storage were found to be 94.4, 81.9, 66.9 and 74.3 for the winter, spring, summer and fall seasons, respectively.

From November 1st until April 1st this milk consistently developed upon storage an oxidized flavor which was readily noticeable 48 hours after bottling by those inexperienced in detecting flavor. The flavor was stronger than that encountered in the grade A milk during the same period. Inasmuch as the irradiated milk had a slightly higher initial ascorbic acid content which disappeared at a greater rate than that in the grade A milk (Table 4), it appeared that the ascorbic acid of this milk was affected by some catalyst not present in the grade A raw milk. Since this milk was irradiated, the natural conclusion would be that the ultraviolet light was exerting the major catalytic effect. However, later work showed that some factor other than irradiation was responsible for the high decrease in ascorbic acid. Copper was evidently responsible as this milk contacted some exposed copper on the spreader trough prior to irradiation. Application of Herrington's test (1) for copper in the metal suspected yielded positive results.

The apparent relation between the percentage decrease of and almost total lack of ascorbic acid and the development of the oxidized flavor seems worthy of note. If ascorbic acid is a reducing substance, then, it appears that appreciable oxidation of the fatty constituents may not be expected in the presence of significant amounts of it. When a distinct oxidized flavor was present, titration showed the presence of little or no ascorbic acid. On the contrary, many samples have been noted in which negligible quantities of ascorbic acid were present, yet there was no development of the oxidized flavor. Such observations were made more frequently during the spring and summer than during the fall and winter. The high stability of summer milk against oxidized flavor development, even with copper contamination within the plant, is well recognized commercially.

Pasteurized milk.—The percentage distribution of the samples of regular pasteurized milk by ascorbic acid content and by season is presented in Table 3. With few exceptions, the regular pasteurized milk throughout the year had a higher ascorbic acid value than did either the grade A raw or the irradiated pasteurized milk, on any of the days of titration. The ascorbic acid content of this milk, as with the other grades, disappeared almost entirely by the fourth day of titration during the winter months. However, the ascorbic acid content of the pasteurized milk during the winter months on the first day's titration was higher than that found in the grade A or in the irradiated milk (Table 4).

The seasonal variations of ascorbic acid in the pasteurized milk, with the exception of during the winter, was not exhibited to any appreciable extent by the results of the first day's titration. These titrations for the winter, spring, summer and fall months gave average ascorbic acid values of 9.9, 12.9, 13.0 and 13.0 mg. per liter, respectively.

The ascorbic acid values obtained for each day's titration, presented graphically in Figure 3, show that the ascorbic acid content of the pasteurized milk was consistently lower in the winter and, after the first day's titration, was lower in the spring than in the summer and fall. Little difference was noted in the amount of ascorbic acid present during these last two seasons.

Of the three milks included in this study, grade A raw, irradiated pasteurized and regular pasteurized, the regular pasteurized was the only kind which did not develop oxidized flavors during the winter season. The milk was pasteurized in glass-lined and in Allegheny metal vats and was conveyed to the bottler through Allegheny metal pipes. The cabinet cooler and distributor were tinned copper, but no exposed copper was noted.

Inasmuch as night's and morning's milk were processed in the forenoon prior to delivery early the following morning, 24 to 34 hours necessarily had elapsed between time of production and time of titration. It must follow, therefore, that the lack of agreement between the lower ascorbic acid values found generally in milk in these studies, grade A, irradiated pasteurized and pasteurized, in comparison with those reported (3, 4, 7) in milk titrated within a few hours after milking, must be due in large part to the factors involved during the lapse of time between the respective titrations.

First days' titrations in these studies represent titration of the milk on the day of distribution, not after it had been held further for another day. Consequently, the ascorbic acid values found on the first titration should represent closely the ascorbic acid value of delivered milk, when the milk is produced and distributed under the conditions of the study.

SUMMARY

Samples of commercial grade A raw, irradiated pasteurized, and regular pasteurized milk, taken daily, bi-weekly, or tri-weekly, at time of delivery throughout the year, were titrated daily for four days for ascorbic acid. The 2–6 dichlorophenolindophenol direct titration method of Sharp (6) was used in determining the ascorbic acid values. On the third day of titration the milk was examined for development of the oxidized flavor.

The ascorbic acid content of winter milk was lower than that of spring, summer, or fall milk on the first, second, third or fourth day of storage. Summer milk under conditions of the study usually had more ascorbic acid than spring and fall milk under similar conditions of study, especially on the third and fourth days. Ascorbic acid values for spring and fall milk generally were between the summer and winter values, but varied as to the period of storage.

The average first day values of ascorbic acid in the grade A milk studied were 6.0, 10.5, 8.8, and 7.7 milligrams per liter during winter, spring, summer and fall, respectively. Those for pasteurized irradiated milk were 7.2, 11.1, 14.2, and 10.9 milligrams per liter while those for regular pasteurized milk were 9.9, 12.9, 13.0 and 13.0 milligrams per liter during the same respective seasons.

The rate of disappearance of ascorbic acid, calculated on the third day of titration, when the milk was examined for oxidized flavor, was greater in the winter than in the summer, being relatively greater in the irradiated and in the grade A milk than in the pasteurized milk. Conversely, the stability of ascorbic acid against oxidation was greater in summer than in winter milk.

The stored irradiated milk had a strong oxidized flavor from late fall to early spring. The grade A raw milk had a less pronounced oxidized flavor during this period, but was yet troublesome, whereas no oxidized flavor was noted in the pasteurized milk throughout the year.

The direct titration method of determining the ascorbic acid in milk was found to be very helpful in predicting the presence of a catalyst in commercial bottled milk.

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MAGNESIUM AND VITAMIN D RELATIONSHIPS IN CALVES FED MINERALIZED MILK

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It has been shown from previous work (1) that dairy calves will develop an anemia when fed whole milk alone which can be prevented by feeding iron and copper salts. It was observed that after these salts were added there still existed deficiencies which appeared after a majority of the calves were 120 to 150 days of age, for they would stand on their toes, with their heads in a lowered position and their backs slightly arched. Many of the bones from these calves were weak, soft, hemorrhaged in areas, and low in magnesium. These observations stimulated further work with calves receiving a limited amount of whole milk supplemented in various ways.

REVIEW OF THE LITERATURE

Experiments which have been done with dogs and rats show conclusively that magnesium is an essential mineral for health and growth (2, 3, 4, 5, 6, and 7).

During the progress of our present experiment, Duncan, Huffman, and Robinson (8) published data showing that calves may succumb to magnesium tetany at almost any time after 30 days of age when fed whole milk alone or supplemented with various combinations of iron, copper, manganese, and cod-liver oil.

Later, Huffman and Duncan (9) fed a group of calves a rachitogenic basal ration and studied the value of adding cod-liver oil, alfalfa hay, magnesium carbonate, and magnesium oxide. Their results showed that when a small amount of vitamin D and a magnesium supplement were incorporated into the rachitic ration rickets did not appear. However, rickets would appear when a magnesium supplement was not fed. They also found, in general, that when calves were fed a rachitogenic ration supplemented with a small amount of cod-liver oil and magnesium the calcium and phosphorus blood picture was maintained at normal up to 10½ months of age.

EXPERIMENTAL

Three groups of three Holstein calves each were used in the present studies. The calves in Group 1 (calves F 549, M 455, and M 480) received daily a basal ration containing a limited amount of whole milk fortified with 400 mg. of iron as iron chloride, and 40 mg. of copper as copper sulfate. Group 2 (calves F 543, M 472, and M 483) received in addition to the basal ration magnesium carbonate at the rate of 1 per cent of the dry matter

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content of the milk fed. The calves in Group 3 (calves F 562, M 478, and M 484) received in addition to the basal ration magnesium carbonate at 1 per cent of the dry matter content of the milk fed and vitamin D from ultraviolet light.

The milk used in this experiment was produced by cows fed alfalfa hay, corn silage or wet beet pulp, and a regular grain mixture. The rate of milk feeding was determined by that consumed by the calves in a previous test (1). This amount varied monthly from 10 to 16.5 pounds per day as the experiment progressed.

Vitamin D was supplied by irradiating the calves for 60 minutes daily with a General Electric S_1 Sunlamp, which was suspended 30 inches above the backs of the calves. The ultraviolet output of the lamps was 10 to 35 microwatts per square centimeter, an amount which had been found sufficient to cure severe rickets in calves fed a rachitogenic ration.

All calves were muzzled throughout the experiment.

Liveweights and measurements of the height at withers were taken at birth and every 2 weeks thereafter. Calcium (10), phosphorus (11), and magnesium (12) contents of the blood serum were determined when the calves were 12 and 13 days of age and at 30-day intervals thereafter. All calves were killed when 255 days of age and the metacarpal, metatarsal, and fifth rib bones removed. All adhering flesh was removed from these bones, and many of them were photographed. The following gross measurements were made of the left metacarpal and metatarsal bones: green weight, length, smallest diameter, width of epiphysis, volume, density, and breaking strength. After breaking, the left leg bones of each calf were ground together and aliquoted. Both fifth ribs from each calf were ground together. All samples were extracted with hot alcohol for 72 hours, and then with ether for 18 hours in Soxhlet extractors. Calcium (13) and magnesium (14) were determined in the bone ash. Phosphorus (15) and bone ash were determined in the moisture-free and fat-free bones.

RESULTS

General Health and Growth

Scours occurred in one or two calves in each group. In M 480 (Group 1) scours was evident for a total of 14 days. M 483 (Group 2) was similarly afflicted for a total of 16 days, and M 478 and M 484 (Group 3) were also afflicted for a total of 46 days. It is evident that scours was not due to magnesium carbonate.

No signs of convulsions were observed.

That M 455 and M 480 (Group 1—basal ration) and M 472 and M 483 (Group 2—basal ration and magnesium) were not normal was indicated by their tendency to stand on the fore part of their front feet. All the calves in Group 3 (basal ration, magnesium, and vitamin D) were normal in this respect.

Growth data given in Table 1 show that from birth to 140 days of age the rate of gain in liveweight was fairly regular. After 140 days of age the rate of gain gradually decreased and was very irregular as the experiment progressed.

TABLE 1

Average rate of growth of calves fed a limited amount of whole milk and various supplements (iron, copper, magnesium, or vitamin D)

Age Days	Group 1 (Whole milk, Fe, and Cu)		Group 2 (Whole milk, Fe, Cu and Mg)		Group 3 (Whole milk, Fe, Cu, Mg and vitamin D)	
	Weight	Height	Weight	Height	Weight	Height
	lb.	*cm.*	*lb.*	*cm.*	*lb.*	*cm.*
1	96	74.49	92	77.14	94	74.06
14	115	75.23	99	77.67	114	74.59
28	123	75.77	116	76.83	121	78.10
42	141	79.58	133	79.14	135	78.51
56	160	81.48	147	81.05	150	80.64
70	179	83.18	173	84.65	168	83.59
84	196	85.09	196	86.13	186	86.13
98	210	86.56	214	89.73	205	87.83
112	230	89.10	234	89.94	219	89.10
126	263	90.57	259	92.91	230	88.56
140	273	92.28	279	94.18	257	92.71
154	280	93.34	296	95.65	280	94.38
168	289	95.45	311	97.36	298	95.88
182	304	96.72	314	98.19	308	96.92
196	316	99.06	322	99.06	334	99.99
210	336	99.69	331	100.73	340	100.96
224	355	99.89	330	103.17	355	102.23
238	358	101.60	360	102.87	373	103.07
255	357	102.87	369	103.50	382	104.97

Blood Data

The data for calcium, phosphorus, and magnesium content of the blood serum (Table 2) show that the calcium values remained fairly normal for most calves throughout the experiment. However, calves M 480 (Group 1) and M 472 and M 483 (Group 2) had blood calcium values low enough to suggest low-calcium rickets. Low-calcium rickets did not appear in Group 3.

The phosphorus content of the blood serum of all calves decreased slightly as the experiment progressed.

The serum-magnesium values for the calves in Group 1 decreased quite rapidly as the experiment continued. On the average the magnesium was below 2.0 mg. when the calves reached 120 days of age. Feeding magnesium carbonate (Groups 2 and 3) maintained the magnesium content of the blood serum at practically a normal level.

TABLE 2

Calcium, phosphorus, and magnesium in 100 cubic centimeters of blood serum of the calves in Groups 1, 2, and 3

Age Days	Calcium		Phosphorus		Magnesium	
	Range mg.	Average mg.	Range mg.	Average mg.	Range mg.	Average mg.
Group 1 (whole milk, iron, and copper)						
12– 13 ...	11.4–11.7	11.56	8.6– 9.3	8.9	2.0–3.9	3.00
34– 45 ...	11.0–11.8	11.29	8.6– 9.0	8.8	2.0–3.1	2.41
67– 74 ...	10.9–12.4	11.50	6.9– 9.2	8.0	2.2–3.0	2.52
95–102 ...	11.3–11.6	11.47	5.7– 8.6	7.2	1.9–2.6	2.25
122–136 ...	10.0–12.4	11.28	6.2– 6.6	6.4	1.7–2.2	1.87
157–168 ...	8.3–11.3	10.20	5.0– 6.1	5.7	1.2–1.4	1.31
172–193 ...	9.3–11.0	10.18	5.8– 6.8	6.3	1.2–1.2	1.20
196–209 ...	8.6–11.8	10.72	5.7– 6.2	6.0	1.1–1.6	1.46
222–234 ...	6.9–11.1	9.57	5.9– 7.2	6.5	1.0–1.5	1.28
246–250 ...	5.6–12.3	9.67	5.6– 6.6	6.2	1.0–1.2	1.12
Group 2 (whole milk, iron, copper and magnesium)						
12– 13 ...	10.0–11.7	10.75	7.4– 9.7	8.6	2.7–4.4	3.37
34– 45 ...	10.3–11.2	10.65	7.3– 8.9	8.2	2.3–2.8	2.57
67– 74 ...	10.4–11.4	10.74	5.7–10.0	8.0	2.4–2.7	2.58
95–102 ...	10.3–11.2	10.65	6.8– 7.5	7.2	2.6–3.7	3.12
122–136 ...	7.9–11.6	9.83	5.9– 8.3	7.2	2.3–2.5	2.41
157–168 ...	7.8–11.4	9.54	5.9– 9.0	7.1	2.1–2.3	2.24
172–193 ..	8.0–10.3	9.02	6.0– 7.0	6.4	2.2–2.4	2.29
196–209 ...	6.8–10.4	8.66	6.5– 6.9	6.6	2.2–2.3	2.28
222–234 ...	7.3–10.8	9.07	6.9– 8.1	7.5	2.0–2.1	2.08
246–250 ...	5.9–11.5	8.74	6.2– 8.0	6.9	1.8–2.5	2.26
Group 3 (whole milk, iron, copper, magnesium and vitamin D)						
12– 13 ...	10.4–11.0	10.65	7.7–10.5	9.1	2.6–2.8	2.66
34– 45 ...	10.1–10.7	10.36	5.2– 7.5	6.6	2.2–2.8	2.49
67– 74 ...	10.6–10.8	10.70	6.4– 8.9	7.3	2.3–2.6	2.47
95–102 ...	10.2–11.2	10.85	5.5– 8.6	7.1	2.5–3.8	2.98
122–136 ...	10.3–10.9	10.57	6.2– 8.1	7.1	2.5–2.7	2.64
157–168 ..	10.2–10.4	10.33	6.6– 7.8	7.2	2.4–2.9	2.59
172–193 ...	9.6–10.5	9.92	6.1– 7.5	6.7	2.3–2.6	2.48
222–234 ...	8.2–10.6	9.71	6.6– 8.4	7.6	2.0–2.6	2.39
246–250 ...	9.2– 9.7	9.44	6.3– 6.8	6.6	2.3–2.5	2.37

Bone Study

The data given in Table 3 show that slight differences existed between the gross measurements of the metacarpal and metatarsal bones of Groups 1, 2, and 3. However, there was a tendency for the bones in Group 3 to be noticeably heavier and somewhat denser than the leg bones in Groups 1 and 2.

Chemical analyses of the metacarpal, metatarsal, and fifth rib bones (Table 4) show that practically no difference in ash, calcium, and phosphorus existed between Groups 1, 2, and 3. The magnesium content of the bones from the calves fed magnesium carbonate in Groups 2 and 3 was 36 per cent higher than that of the bones from the calves in Group 1 (basal ration). The bone-

TABLE 3

Average physical measurements of the left metacarpal and metatarsal bones

Calves	Green weight	Length	Smallest diameter	Width of epiphysis	Volume	Density	Breaking strength*
No.	gm.	cm.	cm.	cm.	cc.		lb.
			Group 1 (whole milk, iron, and copper)				
F 549	225	21.05	2.24	5.62	175	1.28	1601
M 455	242	21.20	2.23	6.00	192	1.26	1422
M 480	273	20.90	2.51	6.12	219	1.24	1511
Av. ..	247	21.05	2.32	5.91	195	1.26	1511
			Group 2 (whole milk, iron, copper and magnesium)				
F 543	219	22.40	2.02	5.44	166	1.31	1619
M 472	252	20.65	2.26	5.62	200	1.26	1244
M 483	254	21.35	2.42	6.10	207	1.22	1325
Av.	241	21.47	2.23	5.72	191	1.26	1396
			Group 3 (whole milk, iron, copper, magnesium, and vitamin D)				
F 562	253	22.20	2.20	5.64	193	1.30	1489
M 478	262	21.10	2.35	6.00	205	1.28	1780
M 484	292	21.20	2.44	6.23	210	1.39	1565
Av.	269	21.50	2.33	5.96	203	1.32	1611

* The base fulcrums of the Tines Olsen Breaking Machine were spaced 4 and 5/8 inches.

magnesium of the calves in Groups 2 and 3 was normal for calves of that age.

The ventral ends of the ribs from the calves in Groups 1 and 2 were enlarged; this is indicative of an insufficient supply of vitamin D. Ribs from the calves in Group 3 were normal in physical appearance (see Fig. 1).

TABLE 4

Chemical analyses of the left metacarpal, metatarsal, and fifth rib bones

Calves No.	Metacarpal and metatarsal				Fifth ribs			
	Ash pct.	Calcium pct.	Phosphorus pct.	Magnesium pct.	Ash pct.	Calcium pct.	Ph pho pc	Magnesium pct.
			Group 1 (whole milk, iron, and copper)					
549	62.86	24.10	11.25	0.3595	61.18	23.51	10.80	0.3414
455	62.47	24.14	11.06	0.2823	60.99	23.51	10.83	0.2903
480	61.19	23.79	10.44	0.3194	55.19	21.15	10.19	0.2836
v.	62.14	24.01	10.91	0.3204	59.12	22.72	10.61	0.3051
			Group 2 (whole milk, iron, copper and magnesium)					
F 543	62.47	23.60	11.78	0.4935	59.82	22.31	11.42	0.4953
M 472	62.47	23.44	11.28	0.4548	59.77	22.34	11.06	0.5475
M 483	61.23	23.41	11.08	0.4776	57.19	21.40	11.19	0.4873
Av.	62.06	23.48	11.38	0.4753	58.93	22.02	11.22	0.5100
			Group 3 (whole milk, iron, copper, magnesium, and vitamin D)					
F 562	64.41	24.62	11.59	0.5149	61.91	23.50	11.25	0.5510
M 478	61.87	23.51	11.70	0.4578	57.14	21.94	10.33	0.4746
M 484	62.83	24.16	11.39	0.4499	58.93	22.43	10.05	0.5150
Av.	63.04	24.09	11.56	0.4742	59.33	22.62	10.54	0.5135

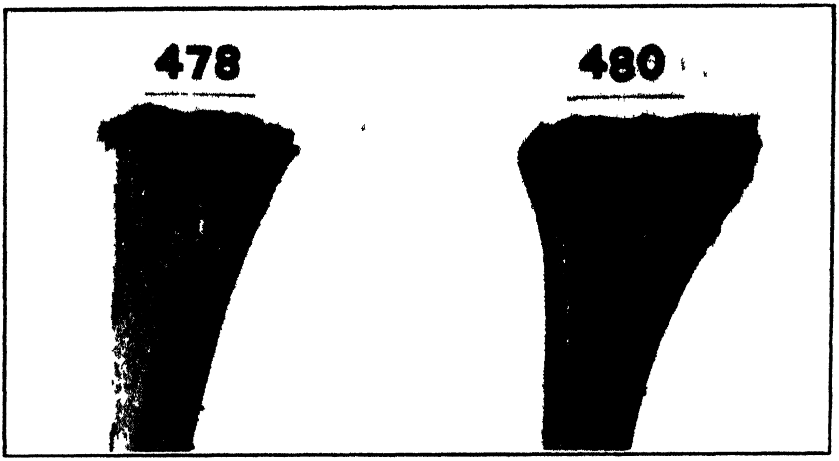

FIG. 1. Costochondral ends of two ribs. Rib 478 is from a calf in Group 3 (whole milk, iron, copper, magnesium, and vitamin D). Rib 480 is from a calf in Group 1 (whole milk, iron, and copper). Rib bones in Group 2 appeared like the ribs in Group 1.

DISCUSSION

This work confirms the observation made in 1932 (1) that low-calcium blood values may be found in calves fed a limited amount of whole milk It is probable that serum-magnesium contents were also low at that time.

Haag and Palmer (2) have shown that a balanced condition of calcium, magnesium, and phosphorus salts in a ration for rats was essential for normal growth and function. Barbour and Winter (4), however, stress the fact that dogs fed a magnesium supplement for a prolonged period of time required an adequate intake of phosphorus in order to prevent a loss of calcium in digestion.

Judging from this work with calves, serum- and bone-magnesium values in calves fed mineralized milk do not appear to be influenced by ultraviolet radiation.

According to Duncan, Huffman, and Robinson (8) all calves would have died in convulsions if they had been kept on the milk, iron and copper ration for a much longer period of time than 255 days.

SUMMARY AND CONCLUSION

Three calves fed a basal ration containing a limited amount of winter milk plus iron and copper salts were compared with three calves fed a like ration supplemented with magnesium carbonate and with three calves fed the basal ration supplemented with magnesium carbonate and vitamin D (ultraviolet light). When magnesium was not added, blood serum-magnesium and bone-magnesium were lowered When magnesium was added, the blood serum-magnesium and bone-magnesium were normal. Ultra-violet radiation produced somewhat heavier bones (green weight, in those calves fed the milk-iron-copper-magnesium ration.

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American Dairy Science Association Announcements

ANNUAL MEETING, JUNE 27–30, 1939

PULLMAN, WASHINGTON, AND MOSCOW, IDAHO

SPECIAL CONVENTION CAR

Members planning to travel to the annual convention by train are advised that the Northern Pacific Railway is planning definitely to provide, for the benefit of the convention party, special cars to be attached to the famous North Coast Limited, leaving Chicago on June 22. See the advertisement of this line in the Journal and note the three plans presented by them for convention travelers and described in detail in an illustrated folder, which may be obtained from the association's Secretary-Treasurer, R. B. Stoltz, of Ohio State University, Columbus, Ohio. The Northern Pacific is known as the Yellowstone Park Line and follows a very scenic route to the convention.

HOUSING

New modern dormitories will be available at both the State College of Washington and at the University of Idaho for use of the members during the convention. The rates will be $1.25 per person per night, for one or two nights. For three nights or more the rate will be $1.00 per person per night. The rate for children will be the same as for adults. The dormitories are close to the meeting places. Rooms will also be available at hotels in both Moscow and Pullman. Reservation of rooms may be made by writing to Professor J. C. Knott, State College of Washington, or Dr. A. O. Shaw, University of Idaho, Moscow, Idaho.

REGISTRATION

Members may register either at the University of Idaho or the State College of Washington, beginning June 26, 1939.

LAST CALL FOR PAPERS

Titles and abstracts of papers must be in the hands of the program committee not later than April 15. Address: Vice-Dean E. V. Ellington, Department of Dairy Husbandry, State College of Washington, Pullman, Washington.

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STUDIES ON THE CONTROL OF GAS IN LIMBURGER CHEESE*[1]

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A problem confronting the makers of limburger cheese is the development of gas in the newly made cheese. In some factories the extent of this gas during the summer is sufficiently great to prohibit the manufacture of limburger and the milk is made into cheddar cheese. During the early stages of ripening of a normal limburger cheese, the body is firm and solid except for a few mechanical holes due to incomplete matting of the curd particles. Depending on the amount of gas present, a gassy cheese may have a few round holes or so many that it resembles a sponge (Figures 1 and 2). A cheese with few gas holes usually develops into a normal cheese, while one badly blown with gas will not ripen normally and is unsaleable for direct consumption.

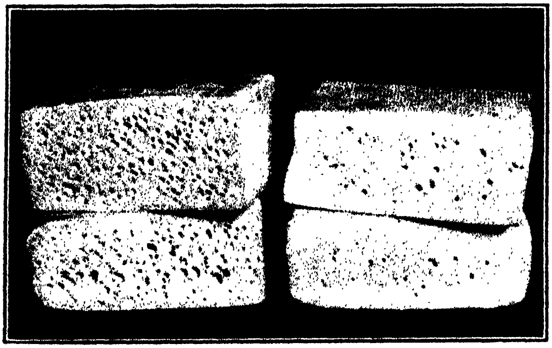

FIG. 1. Upper cheeses made July 7th and the lower ones July 8th. The left hand cheeses were made from raw milk and the right from the same milk heated to 63° C. (145° F.) for 10 minutes.

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* Acknowledgment should be made to the Miller-Richardson Company for aid in gathering material for this investigation, and to the Cherry-Burrell Corporation for the use of a special pasteurizer. The investigation was made possible by the Federal Bankhead-Jones Fund.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 271, May 31, 1938.

These results are concerned with an investigation into the cause of gas in limburger cheese and methods for its control.

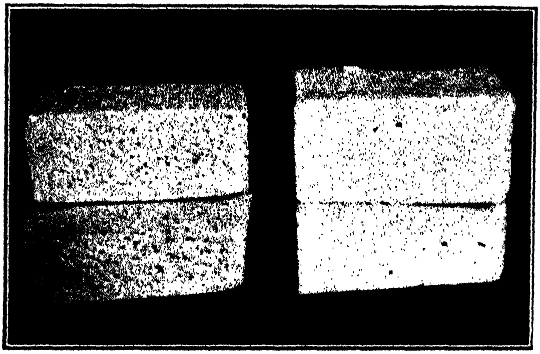

FIG. 2. Lower cheeses made August 4th, the left from raw milk and the right from the same milk heated to 63° C. (145° F.) for 15 minutes. Upper cheeses made August 5th, the left from raw milk and the right from the same milk heated to 63° C. (145° F.) for 20 minutes.

THE CAUSE OF GAS IN THE CHEESE

Five cheeses which eventually developed a large number of gas holes were examined bacteriologically for total numbers of organisms and for numbers of gas-formers of the coliform group. Counts were made of the milk at time of renneting and of the cheese at intervals during the first 24 hours. Total counts were made on Tryptone[2]-glucose agar (Tryptone 0.5 per cent, glucose 0.1 per cent) with the plates incubated for 48 hours at 32° C., while coliform organisms were determined on Leifson's (1) desoxycholate agar plates, incubated 24 hours at 37° C.

Total counts of the raw milk as received during July and August (Table 1) varied from 1,800,000 to 5,000,000,000 per ml. In the milk with the lowest total count 360,000 coliform bacteria were present per ml. and 1,300,000 per ml. in the sample with the highest total number of organisms. The cheese made from these lots of milk showed counts per gram of cheese as high as 600,000,000 in the first instance with 8,000,000 coliform bacteria and 2,200,000,000 in the second instance with 50,000,000 coliform organisms. Although this milk was unsatisfactory for limburger cheese, when milk from the same supply was used in making cheddar cheese, a satisfactory product was obtained. The total counts and ratio of numbers of coliform bacteria to total numbers of bacteria are not greatly out of line for raw milk that has not been under laboratory control; see Harrison et al. (2); Ayers and Clemmer (3).

It is of interest to note that where the heated milk had coliform counts of 21 and 12 per ml. respectively, some gas was to be found in the cheese made

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[2] A casein digest peptone.

TABLE 1

Bacterial counts of gassy limburger cheese during the making process

Sample	Time after renneting	Total count on Tryptone-glucose agar	Coliform count on desoxycholate agar	Ratio coliform count to total count
July 6th	*hrs.*			
Raw milk*	0	240,000,000	150,000	1: 1,600
Heated to 63° C. for 10 min.		6,000	< 100	
Raw cheese[1]	1	240,000,000	< 1,000	
Raw cheese	2	480,000,000	21,000	1: 23,000
Raw cheese	3	110,000,000	180,000	1: 610
Raw cheese	5	600,000,000	180,000	1: 3,300
Raw cheese	8	400,000,000	280,000	1: 1,400
Raw cheese	24	200,000,000	1,100,000	1: 180
July 7th				
Raw milk	0	1,800,000	360,000	1: 5
Heated to 63° C. for 10 min.		132,000	< 10	
Raw cheese	1	...	11,000	
Raw cheese	2	16,000,000	200,000	1: 80
Raw cheese	3¾	20,000,000	2,500,000	1: 9
Raw cheese	5¼	400,000,000	3,000,000	1: 130
Raw cheese	6¾	600,000,000	8,000,000	1: 75
Raw cheese	24	800,000,000	170,000	1: 11,000
July 8th				
Raw milk	0	5,000,000,000	1,300,000	1: 3,800
Heated to 63° C. for 10 min.		88,000	21	1: 4,200
Raw cheese	1	3,400,000,000	580,000	1: 5,900
Raw cheese	2	1,500,000,000	2,000,000	1: 750
Raw cheese	3¼	1,900,000,000	27,000,000	1: 70
Raw cheese	5	2,200,000,000	50,000,000	1: 44
Raw cheese	6¼	2,200,000,000	26,000,000	1: 85
Raw cheese	24	1,600,000,000	4,500,000	1: 350
July 9th				
Raw milk	0	2,900,000,000	7,600,000	1: 380
Heated to 63° C. for 5 min.		14,000,000	12	1: 1,200,000
Raw cheese	1	800,000,000	180,000	1: 4,400
Raw cheese	2	1,300,000,000	130,000	1: 10,000
Raw cheese	3	6,800,000,000	50,000,000	1: 140
Raw cheese	4	1,900,000,000	34,000,000	1: 56
Raw cheese	5	2,000,000,000	74,000,000	1: 27
August 5th				
Raw milk	0	200,000,000	500,000	1: 400
Heated to 63° C. for 20 min.		110,000	< 1	1: 110,000 no gas
Raw cheese	2	980,000,000	300,000	1: 3,300
Raw cheese	3	1,400,000,000	3,400,000	1: 410
Raw cheese	5	1,600,000,000	3,400,000	1: 470

* Counts of milk—colonies per ml.

[1] Counts of cheese—colonies per gram.

from it, while when a longer holding time was maintained and a coliform count was obtained of less than 1 per ml., no gas was found in the cheese.

When the method of manufacture and the temperatures encountered are considered, it is not surprising that where coliform organisms are present a gassy cheese develops. Because it is desirable to keep down the development of acid when making good limburger, it is not the custom to add starter to the milk. While the time and temperature records used during the present study do not necessarily hold true at all times for limburger cheese, these records show how gas forming organisms of the coliform group are encouraged in the making process in general. The milk was heated at once to 30° C. (86° F.) or sligthly higher and rennet added (Figure 3). The temperature was maintained at this point for about 50 minutes when it was raised over a period of about 30 minutes to a temperature of close to 39° C. (102° F.), held for another 30 minutes and then dipped into moulds (Figure 3). For the next 3 or 4 hours, depending upon the weather, the cheeses slowly cooled to 30° C. (86° F.) or lower (Figures 3 and 4[3]) when they were moved

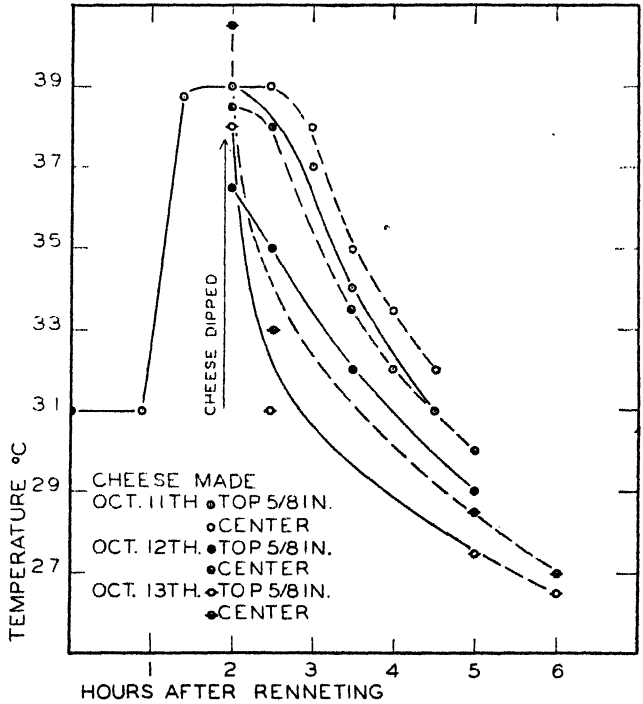

FIG. 3. Temperatures maintained during the making of half pound cheese from the time the rennet was added to the milk until the cheese was placed in the curing room.

[3] The detailed data from which these figures were constructed may be obtained by writing to the Division of Bacteriology, New York State Agricultural Experiment Station, Geneva, New York.

to the ripening room. It will readily be seen that the temperature maintained during the first 5 or 6 hours is more suitable for the growth of coliform bacteria than for the lactic acid bacteria, resulting in rapid increase of gas formers and the production of a gassy cheese. This is confirmed by bacterial counts made during the making process (Table 1), and the change in the ratio of coliform organisms to the total count. The ratio between coliform bacteria and total bacteria was found to increase materially (Table 1) so that the percentage of coliform bacteria was much higher at the end of the day than at the beginning.

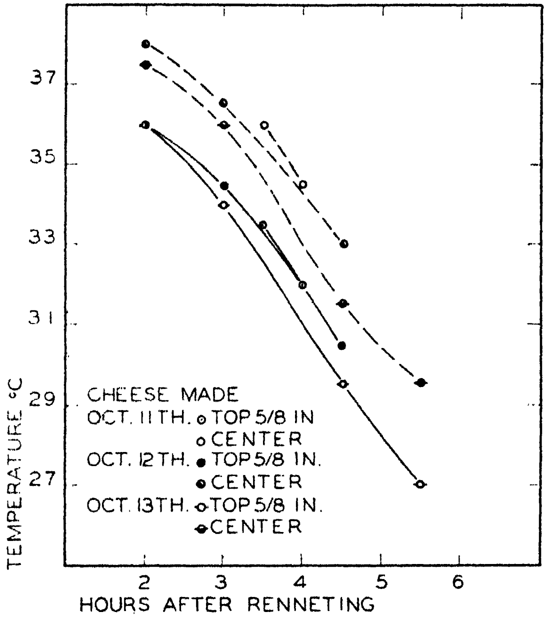

FIG. 4. Temperatures of two pound cheese from the time the curd was dipped into the molds until the cheese was placed in the curing room.

Forty colonies were picked from the desoxycholate agar plates. On further study, 20 cultures were classified as *Escherichia coli* and 20 as *Aerobacter aerogenes*. From this, it would seem that gassy cheese may be produced by either species or by both. As these organisms are commonly found in milk, particularly under certain conditions and at certain seasons of the year, they should be considered a potential source of gas, especially during the summer season.

HEAT TREATMENT AS A MEANS OF CONTROLLING GAS

Zeiler and Berwig (4), in Germany, found they could control gas in limburger types of cheese by heating the milk at 63° C. (145° F.) for 15 or 30 minutes, and make a satisfactory cheese.

During the present investigation heating studies were carried out by three different methods with cheese-factory milk. In the first two cases control cheeses made from the same milk unheated were quite gassy (Figures 1 and 2).

With the first method, 20 gallons of milk were heated in milk cans to 63° C. (145° F.) and held at this temperature for 5, 10, 15, and 20 minutes. The cheeses made from milk held for 5 and 10 minutes showed signs of gas though not as much as the controls, while those made from milk held for 15 and 20

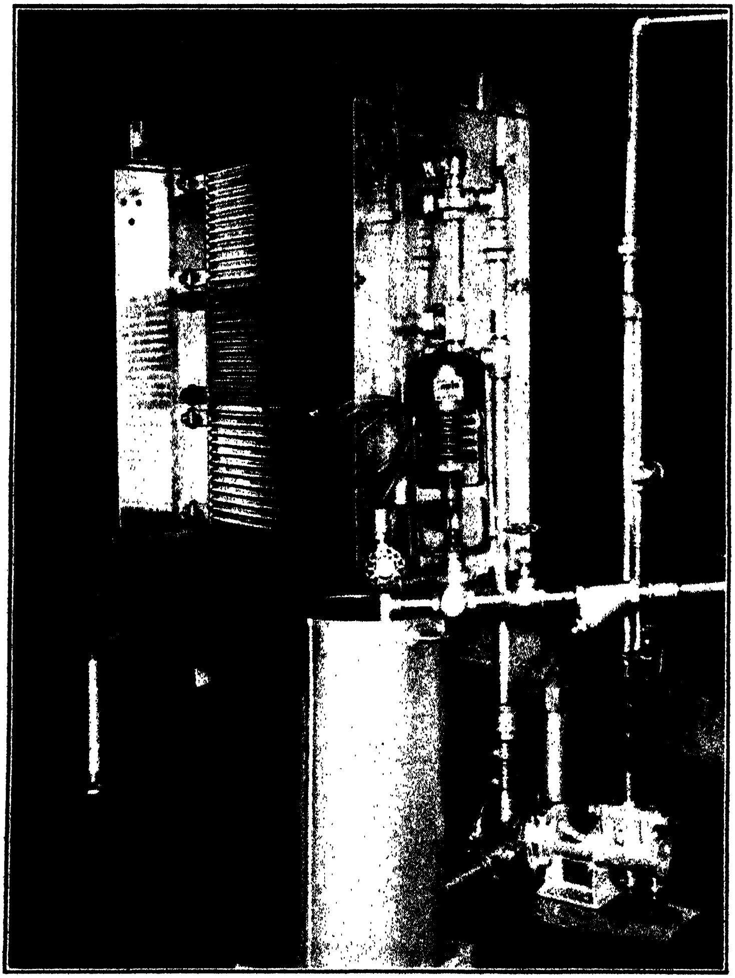

FIG. 5. The pasteurizer used for the second series of experiments. The milk was heated on the upper two sets of coils and cooled on the lower set.

minutes were entirely free of gas (Figures 1 and 2). Cheeses made from this milk were satisfactory in every respect.

For the second series of experiments, a special pasteurizer was furnished by the Cherry-Burrell Corporation (Figure 5) with which the temperature could be controlled within a range of 2 degrees and the milk cooled at once to renneting temperature. The temperature of the water was controlled by a thermoregulator in the tank and the water circulated by a pump through coils. The upper two sets of coils, where the warm water circulated, were used for heating the milk and the lower set for cooling. The milk was fed from a reservoir at the top and ran down over the coils. Temperatures of 70.5°, 81.5°, and 72.5° C. (159°, 161°, and 163° F.) were used during this series and the milk cooled promptly to 30° C. (86° F.). Forty gallons of milk were used in each case. A small amount of gas was found in the cheeses made from milk heated to 70.5° and 71.5° C. though much less than in the raw milk cheeses. The cheeses made from milk heated to 72.5° C. were free from gas. After three months, all of the cheeses made from heated milk were of excellent quality and flavor, while those made from raw milk had pronounced off flavors. The number of experiments carried out are too few to permit definite conclusions to be drawn though they do indicate that heat-

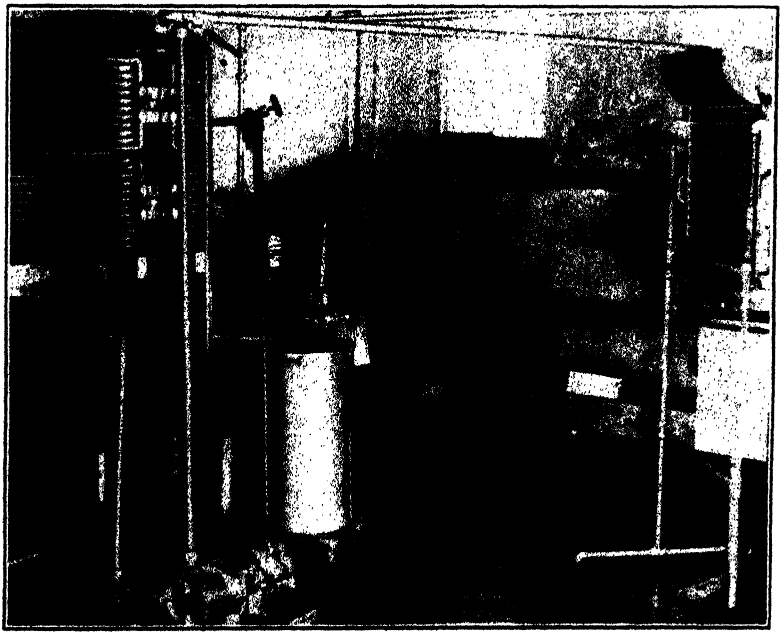

FIG. 6. The pasteurizer used in the third series of experiments. The milk was heated on the two sets of coils, held 15 seconds while passing through the pump and pipes, cooled by the cooler at right and delivered into the cheese vat.

ing to 71.5° C. and cooling immediately is not sufficient to control the gas completely.

In the third series, it was considered advisable to use a time and temperature for pasteurization of the cheese milk commonly accepted by those public health authorities who approve the high-temperature, short-time method for market milk (at least 160° F. for at least 15 seconds). Therefore, the pasteurizer was altered to give a holding time of approximately 15 seconds. This was accomplished by a milk pump, the speed of which could be controlled, at the bottom of the heater with a pipe from the pump to a cooler placed over the cheese vat (Figure 6). Two lots of cheese were made in this series, the milk being heated to 71.0° C. (160° F.) and 72.0° C. (162° F.) and the holding time controlled to 15 seconds by adjusting the speed of the milk pump. In the two trials where this pasteurizing equipment was used the raw milk cheeses were superior to those made from pasteurized milk. It should be pointed out, however, that the methods of making and curing were those practiced in this cheese factory for producing the best quality of cheese from raw milk. Further studies should be made to develop suitable methods for manufacturing pasteurized milk into limburger cheese.

DISCUSSION

As the relatively high temperatures maintained during the period of making, together with the low acidity of the milk, encourage the growth of coliform bacteria, so that gas is produced in the cheese when as few as 12 are present per ml. of milk, it is desirable that milk for making limburger cheese be comparatively free of organisms of this type. The amount of gas produced depends upon the proportion of coliform organisms to other organisms, more gas being produced where the proportion is large than where it is small.

While it is not the purpose of this investigation to suggest heat treatment as the only method of preventing gas in the cheese, it seems a method that can be utilized by the cheese maker where difficulty is encountered due to coliform bacteria in the milk as received from the farm. Systematic grading of the milk as received at the cheese factory would also do much to overcome the problem of gas formation. The desirability of pasteurization as a public health precaution must also be given consideration. This has been more fully discussed in a previous publication (5). Previously published studies at the Station (6) give data dealing with the quality aspect as related to pasteurization. Price (7) found that pasteurization at 62.5° C. (145° F.) for 30 minutes produced a better cheddar cheese than any other method of pasteurization tried.

The present data indicate that a good limburger cheese can be made from milk heated sufficiently to control gas producing organisms. Some feel that a holding method for heating the milk is not as convenient for use in the average cheese factory as a short time method such as was used in the experiments reported here.

When a short time method of heating is used, the equipment can be so arranged as to permit milk to flow from the weight vat through the pasteurizer to the cheese vat. If equipment is used that has adequate time and temperature controls, such pasteurizers would undoubtedly be approved by health authorities if pasteurization of milk for cheese making should be made compulsory.

SUMMARY

Investigations on limburger cheese show that gas in the cheese developed because of the growth of the bacterial species, *Escherichia coli* and *Aerobacter aerogenes*. Even a comparatively small number of these organisms in the milk may cause gas in the cheese. Because the cheese is held at 30° C. or higher for the first six hours, the ratio of coliform organisms to total bacteria present increases rapidly during the period of manufacture.

Pasteurization by either the holding method or the high-temperature short-time method prevents the development of gas. The present studies show that limburger cheese of good quality can be made from milk which has been heated sufficiently to control the development of gas.

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THE INFLUENCE OF HYDROGEN ION CONCENTRATION AND SALT ON THE SURFACE FLORA OF LIMBURGER CHEESE[1]

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Two main types of microorganisms are found in the slime of limburger cheese and assumed to be responsible for the surface ripening (Kelly (1)). Budding yeast-like organisms appear first about the time the cheeses are put on the shelves and increasing rapidly in numbers make the surface of the cheese slimy. Later red pigment-producing, rod-shaped bacteria replace the yeasts and make the slime red and more like butter in consistency.

In speculating as to the factors contributing to the development of the two types of microorganisms to the almost total exclusion of all other types, and the definite sequence with which the rods followed the yeast forms it was thought that salt and hydrogen ion concentration, together with temperature might play a part. Accordingly, cultures of the organisms were isolated and examined as to the influence of these factors on their development.

Macy and Erekson (2) studied the slime of a Roquefort type of cheese and of Port du Salut cheese. Large numbers of *Streptococcus lactis* were found on the surface of the Roquefort cheese before salting, but after salting they disappeared almost entirely and were replaced by yeast forms. As the yeasts increased the pH rose from 4.8 to 5.0. At 21 days the pH was 5.9 and the yeasts started to disappear and were replaced by rods. At 6 weeks the pH was 7.3, red color was found in the slime and torula, rods and cocci as well as *Penicillium roqueforti* were present. In the interior of the cheese the pH was 4.8 when one day old and changed little during six weeks.

The slime of well-developed Port du Salut cheese had a pH of 7.5 to 7.6, with rods predominating. Large numbers of organisms of the *Bacterium linens* type together with some degenerated forms of torula appeared in microscopic preparations. Examinations of the slime of Tilsiter and old Limburger cheeses showed characteristics similar to those of Port du Salut.

INFLUENCE OF pH, SALT, AND TEMPERATURE ON THE ORGANISMS TOGETHER WITH THEIR ACTION ON CERTAIN CARBON AND NITROGEN COMPOUNDS

The cultures of the yeast forms were divided into two main groups, those represented by Cultures 65 (Table 1) growing more rapidly and producing

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TABLE 1

The influence of temperature and hydrogen ion concentration on the growth of yeasts from Limburger cheese

Culture 65							Culture 67						
pH	Reading days	18° C.	25° C.	30° C.	37° C.	45° C.	pH	Reading days	18° C.	25° C.	30° C.	37° C.	45° C.
3.5	1	–	–	–	–	–	3.5	1	–	–	–	–	–
	2	+	+	–	–	–		2	–	–	–	–	–
	3	++	++	–	–	–		3	–	–	–	–	–
	7	++	++	–	–	–		7	+	+	–	–	–
4.5	1	–	–	–	–	–	4.5	1	–	–	–	–	–
	2	+	++	–	–	–		2	–	–	–	–	–
	3	++	++	–	–	–		3	–	–	–	–	–
	7	++	++	–	–	–		7	++	++	–	–	–
5.5	1	–	–	–	–	–	5.5	1	–	–	–	–	–
	2	+	++	–	–	–		2	–	+	–	–	–
	3	++	+++	+	–	–		3	+	+	–	–	–
	7	++	+++	++	–	–		7	++	++	++	–	–
6.5	1	–	+	–	–	–	6.5	1	–	–	–	–	–
	2	++	++	++	–	–		2	–	+	–	–	–
	3	++	+++	++	–	–		3	+	++	–	–	–
	7	+++	+++	++	–	–		7	++	++	++	–	–
7.5	1	–	+	–	–	–	7.5	1	–	–	–	–	–
	2	++	++	++	–	–		2	–	+	–	–	–
	3	++	+++	++	–	–		3	+	+	–	–	–
	7	++	+++	++	–	–		7	++	++	++	–	–
8.5	1	–	–	–	–	–	8.5	1	–	–	–	–	–
	2	–	–	+	–	–		2	–	–	–	–	–
	3	+	+	+	–	–		3	–	–	–	–	–
	7	++	++	++	–	–		7	+	+	+	–	–
9.5	1	–	–	–	–	–	9.5	1	–	–	–	–	–
	2	–	–	–	–	–		2	–	–	–	–	–
	3	–	–	–	–	–		3	–	–	–	–	–
	7	–	–	–	–	–		7	–	–	–	–	–

– = No growth.
+ = First sign of growth.
++ = Good growth.
+++ = Heavy growth.

a heavier pellicle on liquid media than the others represented by Culture 67. In all other respects the cultures appeared the same and are probably variants of one species.

As stated before (1) these yeast forms were found to reproduce by budding, showed no trace of sexuality, did not produce spores, formed a pellicle on liquid media from the beginning, and tolerated salt in concentrations as high as 18 and 20 per cent. They showed their best growth at a temperature of 25° C. and a pH about 6.5, though growth was observed from pH 3.5 to 8.5 (Table 1). Fructose, glucose, mannose, galactose, sucrose, maltose, lactose, raffinose, ethyl alcohol, and sodium and calcium lactate were utilized as carbon sources with the production of carbon dioxide.

Both Bacto proteose peptone and Bacto peptone were readily utilized as a source of nitrogen without any other source of carbon. A proteose peptone (3), made by salting out with ammonium sulphate and dialyzing the precipitate to get rid of the sulphate, was able to support growth in the presence of a fermentable carbohydrate but not without. Ammonium sulphate, ammonium nitrate, ammonium phosphate, and asparagin could not be used as a sole source of nitrogen in the presence of a fermentable carbohydrate.

TABLE 2

The influence of temperature and hydrogen ion concentration on the growth of Bacterium linens

Cultures 4 and 56a—*Bacterium linens*

pH	Reading days	18° C.	25° C.	30° C.	37° C.	45° C.
3.5	2	–	–	–	–	–
	3	–	–	–	–	–
4.5	2	–	–	–	–	–
	3	–	–	–	–	–
5.5	2	–	–	–	–	–
	3	–	–	–	–	–
5.85	2		+			
	3		+			
6.0	2		+			
	3		+			
6.5	2	+	+	+	–	–
	3	++	++	++	–	–
7.5	2	+	+	+	–	–
	3	++	++	+	–	–
8.5	2	+	+	+	–	–
	3	++	++	+	–	–
9.5	2	+	+	+	–	–
	3	++	++	+	–	–

– = No growth.
+ = Small amount of growth.
++ = Good growth.

Gelatin was not liquefied, and a slight alkaline reaction was noted in litmus milk with reduction of the litmus at the bottom of the tube.

At the present time no attempt is made to classify these yeast forms.

The rod forms are represented by Culture 4, isolated in the present study, and Culture 56a (Table 2), obtained from Kiel. Both are typical *Bacterium linens* Weigmann.

It is to be noted that Culture 4 and other cultures isolated in this study produced the typical orange-colored ring in milk in contrast to that previously reported (1).

Bacto peptone was readily utilized both as a carbon and nitrogen source, but asparagin, ammonium sulphate, and ammonium nitrate could not be used. No apparent action was noted on any of the carbohydrates. The optimum temperature for growth was about 25° C. with no growth at 37° C. (Table 2). A hydrogen ion concentration of pH 6.5 proved most satisfactory with growth at pH 9.5 and as low as 5.85 but not lower (Table 2). Salt in concentrations as high as 18 and 20 per cent was tolerated in both solid and liquid media.

In order to correlate the findings in the laboratory with conditions in factory cheeses, hydrogen ion determinations together with salt determinations were made on cheeses in the factory varying in age from the new cheese to those ready for market.

METHODS

The method previously employed of sampling factory cheeses, varying in age by a day, was largely employed in the present studies. Where possible the same batch of cheeses were sampled from day to day.

In sampling the cheeses, the outer slimy surface was scraped off with a knife and labelled "A." After this five-sixteenth of an inch of cheese was cut off the outside and called sample "B." The next five-sixteenth of an inch was called "C" and the center "D." Salt, hydrogen ion and in some cases moisture determinations were made of these portions. At the same time bacterial samples were taken of the surface by bringing a glass slide in contact with the surface slime (1).

Hydrogen ion determinations were made with the quinhydrone electrode following the method of Sønke Knudsen as modified by Watson (4). Salt was determined in duplicate by the method recommended by Wilster *et al.* (5), for hard cheese. Though Limburger cheese is classed with the semisoft cheeses it is firm in the early stages and the method proved quite satisfactory.

In all, six lots of cheese were examined for hydrogen ion concentration and four lots for salt, covering periods of May, July, and October.

RESULTS

Hydrogen Ion Changes During Ripening

The pH dropped from about 6.45 in the milk at time of renneting to below 5.0 in the cheese within a few days (Tables 3, 4, 5 and Figure 1).

TABLE 3

Changes in hydrogen ion concentration of Limburger cheese made in May

Description of cheese	Age days	pH of surface	Microorganisms in surface smears
	2	5.64	Few bacteria and yeasts
	3	5.81	Yeasts increasing
	4	5.95	Yeasts in masses, a few rods
	5	6.57	Yeasts in masses, a few rods
First color	6	6.84	Yeasts in masses, a few rods
Good color	7	6.95	Yeasts in masses, a few rods
	8	7.56	Masses of yeasts and rods
	9	7.50	Masses of yeasts and rods
	10	7.75	Masses of rods, a few yeasts
Ready for storage	11	7.85	Masses of rods, a few yeasts

After the first or second day when the yeasts started to grow the pH of the surface slime was found to rise to around pH 7.0 or higher at about 7 to 12 days when the cheeses were ready to go to storage. Because of lower temperatures in the ripening room, the pH of the October cheeses did not go above 6.7 before being transferred to storage. The recorded figures in some cases may be somewhat low as difficulty was encountered in scraping off the slime without getting some of the interior of the cheese. This factor together with the gas in two cases would no doubt explain the irregularities of the points of the curves designated surface and 1st 5/16th in. (Figure 1). Little further change was noted in the pH of the surface up to the time the cheese was ready for market.

In the interior of the cheese (Samples B, C, and D) the rise in pH was not as rapid and hardly reached the neutral point at any time during ripening, being around pH 6.9 when ready for market. The rods (*Bacterium linens*) did not appear until the pH of the slime was around 5.85 and usually when it was higher. The red color appeared when the surface smear was somewhere about pH 6.8. When *Oospera* were found in any numbers in the surface smear or the cheese was gassy the pH was invariably lower than normal (Table 4, Figure 1).

The desirability of this first drop in pH, due no doubt to the growth of lactic acid streptococci, was shown in some work carried out on cheeses made at this same time from milk pasteurized at 143° F. for 30 minutes. Two lots of cheese were made with the milk as received and the pH did not drop below pH 6.0 (Table 6) followed by a steady rise in pH. When these cheeses were examined at a later date they were found to have a strong putrid odor. Another lot was made from milk of the same supply but 2 ml.

TABLE 4

Changes in hydrogen ion concentration of Limburger cheese made in July, 1937

Description of cheese	Age	pH in cheese				Microorganisms in surface smear
		Surface	First 5/16 inch	Second 5/16 inch	Center	
White slime	1 day	4.95	4.90	4.80	4.65	A few rods evenly scattered
	2 days	5.05	4.90	4.75	4.80	A few yeasts
	3 "	4.95	4.85	4.65	4.70	Fewer rods, yeasts well distributed
	4 "	6.05	5.20	4.70	4.70	Yeasts, some rods increasing
	6 "	6.45	5.45	4.75	4.60	Yeasts in masses, rods fewer
Gassy cheese	7 "	5.70	5.00	4.50	4.70	Yeasts more numerous, rods fewer
Good red color	8 "	7.00	5.70	4.80	4.70	Yeasts same, rods in masses
Gassy cheese	10 "	5.75	5.20	4.75	4.70	Yeasts fewer, rods fewer. *Oospera* present
	11 "	7.25	5.20	4.75	4.70	Yeasts few, rods in masses, a few *Oospera*
	12 "	7.35	5.35	4.85	4.70	Yeasts fewer and smaller, rods in masses
Ready for storage	13 "	7.40	5.60	4.80	4.65	Yeasts in masses but small, rods in masses
	4 weeks	7.35	5.35	4.85	4.70	Yeasts small, rods in masses
	5 "	7.35	6.00	5.45	4.90	No yeasts, rods in masses
	6 "	7.35	6.25	5.55	5.10	Yeast small and numerous, rods in masses
	7 "	7.25	7.00	6.05	5.55	Yeast small and few, rods in masses
Ready for market	8 "	7.45	6.25	6.05	5.80	Yeasts small and few, rods in masses

TABLE 5

Changes in hydrogen ion concentration of Limburger cheese made in October, 1937

Description of cheese	Age	pH in cheese[1]				Microorganisms in surface smear
		Surface	First 5/16 inch	Second 5/16 inch	Center	
	4 hours		5.65			
	6 "		5.33			
	1 day	4.37	4.37	4.30	4.25	Few bacteria and yeasts
	2 days	4.30	3.95	3.95	3.95	Yeasts increasing
	3 "	4.97	4.75	4.80	4.80	Yeasts the same
	3 "	4.82	4.70	4.70	4.70	Yeasts in masses
	4 "	4.70	4.55	4.25	4.50	Yeasts in masses
First red color	6 "	5.25	4.61	4.60	4.55	Yeasts and rods in masses and some *Oospera*
	7 "	5.20	4.90	4.65	4.50	Yeasts and rods in masses and some *Oospera*
	8 "	5.55	4.65	4.60	4.50	Yeasts and rods in masses and a few *Oospera*
	9 "	5.60	4.85	4.65	4.50	Yeasts and rods in masses and a few *Oospera*
	10 "	5.20	4.90	4.75	4.66	Yeasts and rods in masses and a few *Oospera*
	11 "	6.70	5.40	4.75	4.65	Yeasts and rods in masses
Ready for storage	12 "	6.55	5.10	4.60	4.60	Yeasts and rods in masses

The first 6 samples were from cheeses made October 4th. The remaining samples were from cheeses made October 1st.

[1] Cheese made from milk with pH 6.45 at the addition of rennet.

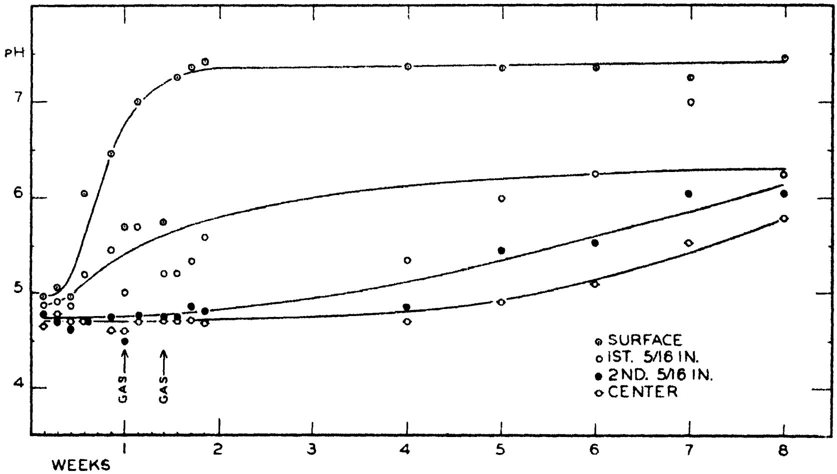

FIG. 1. Changes in hydrogen ion concentration of Limburger cheese made in July, 1937.

of starter were added for each gallon of milk at the time of renneting. By the third day the pH had dropped to 5.0 and though this drop was not as rapid or as low as in many commercial cheeses a typical Limburger developed with no sign of the putrefaction found in other cheeses. This development of acid appears to be necessary to control putrefying bacteria in Limburger cheese as in the hard pressed varieties.

Concentration of Salt in the Cheese

As would be expected and as shown by Mrozek (6), Byers and Price (7), and others the salt in the cheese was found in greatest concentration in the surface layers and lowest in the inner portions soon after salting (Tables 7, 8, and 9 and Figure 2). On the first day the percentage of salt was between 3.13 and 3.30 in the surface layer (Sample A), 2.03 to 3.04 in the second layer (Sample B), 0.36 in the next layer (Sample C) and 0.06 to 0.31 in the center (Sample D). From this time on the salt tended to diffuse

TABLE 6

Influence of added starter on hydrogen ion concentration and quality of Limburger cheese made from milk pasteurized at 143° F. for 30 minutes

	Age in days						
	½	1	2	3	4	6	7
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
No starter added. Cheese became putrid	6.0	6.15	6.30		6.30	6.30	
No starter added. Cheese became putrid	6.25		6.15		6.65		
Starter added. Good cheese	6.15		5.55	5.00			6.00

through the cheese until an equilibrium was reached near the tenth day when the percentage was about the same throughout the cheese. Considering that the method of salting is by rolling the cheese in the salt and rubbing with the hands it is surprising to find the salt content from cheese to cheese as uniform as it is. Though moisture should have been taken into consideration in reporting the salt content it was difficult to obtain satisfactory samples. However when corrections were made using the moisture determinations that were made the variations in salt content were so small as not to change greatly the relationship of the amounts as given (Tables 7, 8, and 9 and Figure 2).

TABLE 7

Changes in salt content of Limburger cheese made during July, 1937

Age	Surface	First 5/16 inch	Second 5/16 inch	Center
1 day	3.28	3.04	0.63	0.31
2 days	3.12	2.71	1.15	0.61
3 "	2.33	2.33	1.64	0.80
4 "	1.56	2.31	1.60	0.93
6 "	2.33	2.34	2.00	1.56
7 "	2.38	2.23	1.68	0.91
8 "	1.55	2.05	1.71	1.41
10 "	2.10	2.23	2.21	1.71
11 "	1.69	1.85	2.13	2.10
12 "	2.56	2.46	2.53	2.40
13 "	2.00	2.53	2.36	2.13
4 weeks	1.49	1.69	2.03	1.76
5 "	1.46	1.80	1.78	1.73
6 "	1.46	1.70	1.80	1.90
7 "	1.81	1.88	1.80	1.70
8 "	1.88	1.96	2.11	2.09

TABLE 8

Changes in salt content of Limburger cheese made during October, 1937

Age days	Surface	First 5/16 inch	Second 5/16 inch	Center
1	3.13	2.03	0.90	0.30
2	2.15	1.20	0.88	0.55
4	2.03	1.90	1.40	1.26
5	1.76	1.73	1.73	1.50
7	1.66	1.96	1.86	1.56
9	1.64	1.68	1.48	1.42
11	1.46	1.43	1.53	1.46
12	1.66	1.56	1.66	1.43

In determining the influence of salt on the growth of bacteria in cheese several factors must be taken into consideration in estimating the concentration of salt in contact with the organisms. It is to be expected that most of the salt would be dissolved in the moisture of the cheese and because of this the percentage of salt as determined by an analysis of the cheese does

TABLE 9

Changes in salt content of Limburger cheese made during October, 1937

Age days	Per cent of salt			
	Surface	First 5/16 inch	Second 5/16 inch	Center
1	3.30	2.86	0.36	0.06
2	2.26	2.13	1.50	0.36
3	2.30	2.23	1.86	0.76
3	2.13	1.82	1.44	1.02
4	1.80	1.76	1.50	1.03
5	1.60	1.46	1.70	1.06
6	1.53	1.56	1.54	1.15
7		1.76	1.60	1.50
8		1.76	1.60	1.46
9		1.73	1.60	1.53
10		1.76	1.63	1.46
11		1.76	1.60	1.56
12		1.76	1.63	1.53

not give a true picture of conditions in the cheese. Therefore in estimating the salt content of the medium in which the bacteria are growing the moisture content of the cheese must be taken into consideration. The moisture content of the surface layer varied from 40 to 42 per cent and from 42.5 to 44 per cent in the center portion. Assuming that all the salt is in solution in the cheese moisture, this brine in the surface layer of a one-day-old cheese

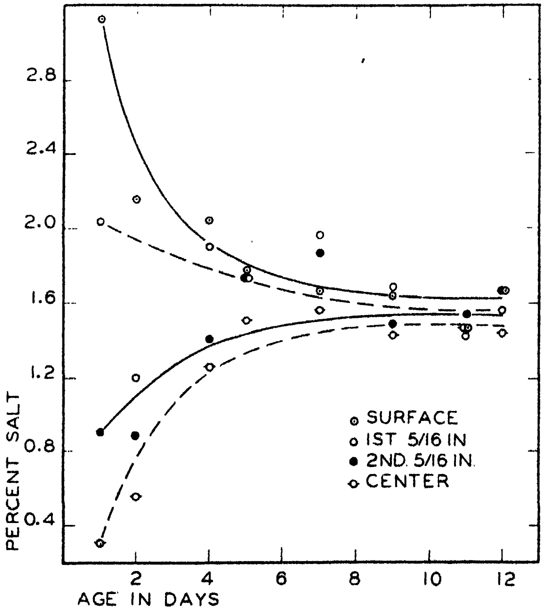

FIG. 2. Changes in concentration of salt in Limburger cheese.

(Table 9) with 41 per cent moisture would have 8.0 instead of 3.3 per cent salt. At 2 days the per cent of salt would be 5.5 instead of 2.26 and at 5 days 3.9 instead of 1.6 per cent.

McDowall and Dolby (8), working with Cheddar cheese, came to the conclusion that a large part of the cheese moisture was not free moisture though they were unable to make conclusive tests. However they estimated that 20 to 30 per cent of the moisture in a young cheese was bound. Assuming that 30 per cent of the moisture is bound, the concentration of salt in the free water would be 11.4 per cent in the surface layer of a day-old cheese. The surface layer of the 2-day-old cheese would then have a salt content of 7.8 per cent and the cheese at 5 days, 5.5 per cent. These theoretical considerations establish that the salt concentration of the cheeses a few days after salting would in no way retard the growth of the organisms desired.

A short time after the cheese is salted, on the evening of the day when made and the morning of the following day, the salt content of the brine in the surface layer must be near the saturation point as there is a considerable amount of undissolved salt present. Therefore for at least some hours after salting the cheese, only salt tolerant organisms would multiply. This is given support in the work of Macy and Erekson (2) where it was shown that *Streptococcus lactis* disappeared when the cheese was salted and yeasts took their place.

DISCUSSION

During making and the early stages of ripening the rapid fall in pH is undoubtedly largely due to the action of lactic acid streptococci. The pH which falls lower than in normal Cheddar cheese is no doubt due to a higher moisture content in the Limburger cheese with more lactose to be converted to lactic acid. This lowering of the pH is desirable in making Limburger cheese in that it keeps putrefying bacteria from growing.

The high concentration of salt on the surface suppresses the many miscellaneous organisms found on the new cheese and permits the development of the yeasts that can grow at a low pH. The yeasts by acting on both the lactate and the protein soon raise the pH above the point (pH 5.85) where *Bacterium linens* can start growth. Other organisms that might grow at this pH are suppressed by the concentration of salt while *Bacterium linens* grows readily. At this point the yeasts have completed their work and soon die out, being overgrown by *Bacterium linens*. Acting on the protein *Bacterium linens* raises the pH still higher and is believed to complete the ripening of the cheese. The cheeses become inoculated with both organisms from the ripening shelves, which in the factory are heavily seeded with the organisms.

Though no work as yet has been carried out on the internal flora of the cheese it does not seem probable that *Bacterium linens* would be a factor because of its aerobic nature and its inability to grow at a pH lower than 5.85. This tends to strengthen the theory that the soft buttery texture and a part of the flavor is produced by the action of enzymes of *Bacterium linens* rather than by direct action of the organism itself. The question whether the enzymes penetrate the cheese and digest proteins could best be established by a study of the enzymes.

SUMMARY

The yeast forms first appearing on the surface of Limburger cheeses will grow readily on artificial media between pH 3.5 and 8.5 and with the best growth at pH 6.5.

Bacterium linens will not grow below pH 5.85 but will grow at pH 9.5 and grows best at pH 6.5.

In the cheese the pH of the surface falls to 5.0 or lower, a pH at which the yeast will grow but not *Bacterium linens.* The yeasts acting on both the protein and lactic acid, raise the pH above 5.85 at which point *Bacterium linens* can become established and overgrow the yeast. Only late in the period of ripening does the pH of the interior of the cheese rise above 5.85. This pH and the lack of oxygen would inhibit the growth of *Bacterium linens* in any part of the cheese excepting the surface.

The concentration of salt soon after salting is highest in the surface layer and low in the interior. There is a gradual penetration of salt into the cheese until an equilibrium is reached at about 10 days. With the lack of information on how much of the moisture of the cheese is available to form brine it is impossible to estimate accurately the concentration of salt in the medium in which the bacteria are growing. However, as the cheeses are salted by rubbing salt on the outside there is little doubt but that it is near the saturation point in the surface layer for some time. This high concentration of salt tends to inhibit the growth of organisms other than the yeasts and *Bacterium linens.*

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CAROTENE IN THE NUTRITION OF DAIRY CALVES*

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The necessity of vitamin A for proper growth and well being of dairy calves has been conceded quite generally for the past ten years. It has been shown that for this species the general symptoms of vitamin A deficiency are slow growth, general unthriftiness, scours, blindness, susceptibility to pneumonia, and oedema.

Studies at the Pennsylvania Agricultural Experiment Station in which large amounts of cottonseed meal were fed to calves have shown definitely that supplements of cod liver oil or alfalfa hay of good quality will prevent the usual malnutrition or cottonseed meal injury that results from such feeding practice. If the injury had not progressed too far cures were affected by the same treatment. The vitamin A present in the cod liver oil and the carotene (the mother substance of vitamin A) in alfalfa undoubtedly played a major rôle in the prevention and cure of this malnutrition. Carotene dissolved in cottonseed oil, on the other hand, apparently had little or no effect in the prevention or cure of this nutritional ailment.

Jones, Eckles and Palmer (11) showed very conclusively that vitamin A is indispensable in the growth of dairy calves. Bechdel, Eckles and Palmer (3) in the same year reported that calves developed symptoms of vitamin A deficiency when cod liver oil was not included in their experimental ration of corn gluten feed, casein, polished rice and butterfat.

Converse and Meigs (6) found that cows fed on timothy hay of low grade for a period of two or more years were unable to produce normal calves. Calves fed on the milk from these cows soon died. The investigators found that vitamin A in the form of carotene or cod liver oil can be added to the deficient milk rations of the calves and by doing so make them adequate for producing approximately normal growth.

After depleting calves of their body store of vitamin A by feeding a basal diet composed of skimmilk, cottonseed meal, dried beet pulp and bone meal, Kuhlman, Gallup and Weaver (12) found that 5 ml. of a commercial carotene preparation fed in combination with a vitamin D supplement was as effective in relieving the vitamin A deficiency symptoms as 30 ml. of cod liver oil.

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Bechdel (2), and Bechdel, Williams and Skaggs (4) produced typical cottonseed meal injury in calves. The symptoms simulated vitamin A deficiency, only the injury seemed to be somewhat more severe in that occasional cases of convulsions were observed. Cod liver oil gave adequate protection against the injury and brought about rapid recovery, while carotene seemed to be less effective in protecting against the cottonseed meal injury.

Hart and Guilbert (9) observed vitamin A deficiency in cattle under range conditions. They report (10) that about twenty-nine micrograms of carotene in alfalfa hay per day per kilogram of body weight would prevent or cure vitamin A deficiency symptoms in cattle.

Investigators have shown that the type of solvent used is an important factor in determining the biological value of carotene. According to Dutcher, Harris, Hartzler and Guerrant (7) carotene dissolved in mineral oil cannot be utilized by the rat.

Moore, Huffman and Duncan (14) observed blindness in calves dropped by cows while receiving roughage of poor quality. These investigators observed a similar condition among growing heifers fed the same type of ration. Kuhlman, Gallup and Weaver (13) reported two types of blindness among calves which may be related to vitamin A deficiency.

EXPERIMENT I

Grade Holstein calves were used as experimental subjects. The concentrate mixture fed was similar to calf rations commonly used on good dairy farms. In order to keep the vitamin A potency of the ration quite low, yellow corn was not included in the mixture. The constituents were as follows:

Wheat bran	150 pounds
Oat mids	200 pounds
Hominy feed	300 pounds
Linseed meal	100 pounds
Distillers' grain	50 pounds
Irradiated yeast	1 pound
Salt	2 pounds

The treatment of the calves previous to going on experiment consisted in feeding them whole milk until twenty days of age. At this time the whole milk was gradually replaced with skimmilk which was fed to all calves continuously while on experiment. Timothy hay of very low grade, almost devoid of green color, was fed prior to as well as in the experimental period. The skimmilk was produced by cows fed on a low vitamin A diet.

All animals were put on the basal ration at the beginning of the experiment, and no change was made until symptoms of vitamin A deficiency were observed. The symptoms first noticed were loss of appetite and scouring. This was followed by a general drop in body weight and blindness developed in two of the five animals used. The weight of the animal was the main criterion used in determining when to start supplementing the ration with the treatment.

A preparation of carotene dissolved in cottonseed oil was used as the source of vitamin A for two animals (calves 338 and 340). This preparation contained approximately 3,000 U.S.P. units of vitamin A activity per gram. The amounts of this supplement fed are given in Figure 1. It was fed daily by adding it to the skimmilk just before feeding. Two animals (calves 337 and 341) were fed two pounds of fresh carrots daily containing approximately 30,000 vitamin A units. Dehydrated alfalfa hay of good quality was fed to one calf (calf 342).

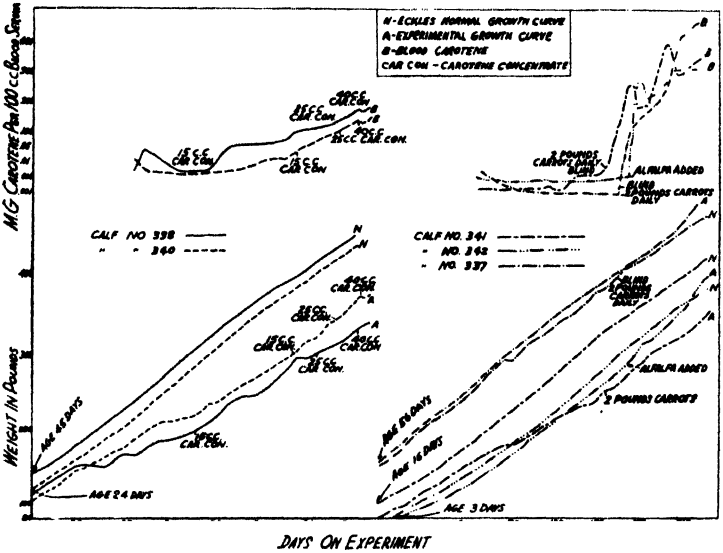

FIG. 1. Growth and blood carotene curves of calves used in Experiment I.

Carotene content of the blood serum was determined at regular intervals throughout the experimental period. The Palmer method modified by Conner (5), and White and Gordon (15) was used. Non-protein nitrogen, glucose and hemoglobin were determined on the blood of each animal at regular intervals.

RESULTS ON INDIVIDUAL CALVES

After the animals were depleted of their vitamin A reserve they were treated as individuals and no effort was made to experiment with them as groups or pairs.

The treatment and response of each animal is portrayed in Figure 1. The Eckles normal growth curve is used as a standard for growth.

Calf No. 338 which had received the carotene concentrate made normal growth for only the first 30 days on the experimental ration. It developed pneumonia on the 44th day and from that date on showed typical symptoms of vitamin A deficiency. Carotene supplement in large doses was administered on the 109th day and continued until the end of the experiment. This amount of carotene was effective in increasing the blood carotene slightly but the animal's growth rate gradually declined.

Calf No. 340 which also received the carotene concentrate made normal growth on the experimental ration for the first 85 days when typical symptoms of vitamin A deficiency, scours, loss of appetite and nasal discharges appeared. The administration of the carotene supplement did not increase the blood carotene or the growth rate to an appreciable extent.

Calf No. 337 made normal growth on the experimental ration for the first 81 days. It showed typical vitamin A deficiency symptoms, consisting of scours, loss of appetite and finally blindness on the 164th day. When carrots were fed, the blood carotene increased rapidly and the growth rate was accelerated.

Calf No. 341 was below normal in weight when put on experiment. Its growth rate remained almost constant for the first 80 days, but declined appreciably from this time on until 156 days on experiment. Blindness was observed on the 142nd day. About one week after carrots were added to the ration the growth rate began to increase. A phenomenal increase of the blood carotene was also observed after carrots were fed.

Calf No. 342 made practically normal growth gains although she showed symptoms of vitamin A deficiency such as scours, loss of appetite and nasal discharges. The addition of alfalfa to the ration increased the blood carotene immediately, and the rate of growth was also increased remarkably. Other symptoms of vitamin A deficiency also disappeared promptly.

GENERAL DISCUSSION

The results with calves Nos. 338 and 340 indicate that carotene dissolved in cottonseed oil is not utilized to the extent that it will prevent or cure avitaminosis A that results from feeding the above experimental rations. There is evidence that the calves utilized some of the carotene but not enough to make its effect significant.

Carrots were quite effective in correcting the avitaminosis A (blindness excepted) in calves Nos. 337 and 341. Alfalfa hay of good quality (dehydrated) appeared to be slightly more effective than carrots as shown by the results on calf No. 342. This is explainable through the fact that the alfalfa was fed in relatively large amounts and, therefore, more vitamin A units were supplied than in the carrots fed to the calves Nos. 337 and 341.

The blood carotene studies are of special interest in this connection. The chart reveals that the carotene appeared in the blood stream almost immediately in large amounts when carrots or alfalfa were fed to the calves suffering from avitaminosis A. The blood carotene was increased in amounts that were barely measurable when a carotene supplement was fed to the calves suffering with avitaminosis A. It is interesting to note, as indicated in the chart, that the potential units of vitamin A in the carotene supplement administered were much greater than the total unitage contained in the carrots.

Non-protein nitrogen, hemoglobin, and sugar determinations made during the depletion period did not show abnormal values as found by Anderson, Gayley and Pratt (1).

EXPERIMENT II

A second experiment was planned with the view of obtaining further information on the effectiveness of carotene in preventing and in curing the usual avitaminosis A that occurs from feeding calves on hay of poor quality. Timothy hay of low grade (with little green color) was used in this experiment. Six calves (calves 354, 356, 357, 361, 362, and 363) were fed a concentrate ration low in vitamin A. This grain ration was very similar to rations commonly used by dairy farmers. Five other calves (calves 349, 350, 355, 364, and 365) were fed a grain ration which contained fifty-seven per cent cottonseed meal. Two of these calves (calves 364 and 365) received no hay. All calves were left with their dams for two or three days after birth. They were then fed whole milk until two weeks of age, after which skimmilk was gradually substituted for the whole milk. All calves were weighed at weekly intervals at which time blood samples were taken for carotene determinations.

The timothy hay, assayed by the colorimetric method, was found to contain 4.5 micrograms of carotene per gram. The carotene supplement was guaranteed to have a biological activity of approximately 3,000 U.S.P. units of vitamin A per gram. This concentrate was weighed into ¼ and ½ ounce capsules and administered by means of a balling gun. The livers of all calves that were slaughtered were assayed for vitamin A by the antimony trichloride method as outlined by Guilbert and Hart (8).

One of the calves (calf 362) receiving the low vitamin A ration was fed four and one-half grams of the carotene supplement beginning with the sixth week and continuing to the end of the experiment. The five other calves were fed the basal ration until marked symptoms of vitamin A deficiency occurred. At this time amounts ranging from four and one-half to eighteen grams of the carotene supplement were administered daily to the different individuals (Figure 2). Three of the calves (calves 349, 350, and 355) receiving the high cottonseed meal ration were treated in a like manner. One of the two calves (calf 364) receiving no hay was given four and one-half grams of the carotene supplement daily. The other calf (calf 365) was continued on the basal ration until it became very weak. Nine grams of the carotene supplement were then administered to this animal daily. Here again, the chief criterion for starting the administration of carotene supplement was failure of the animal to gain in weight. Other symptoms of vitamin A deficiency, especially dilated pupils and blindness, were used as further means of diagnosis.

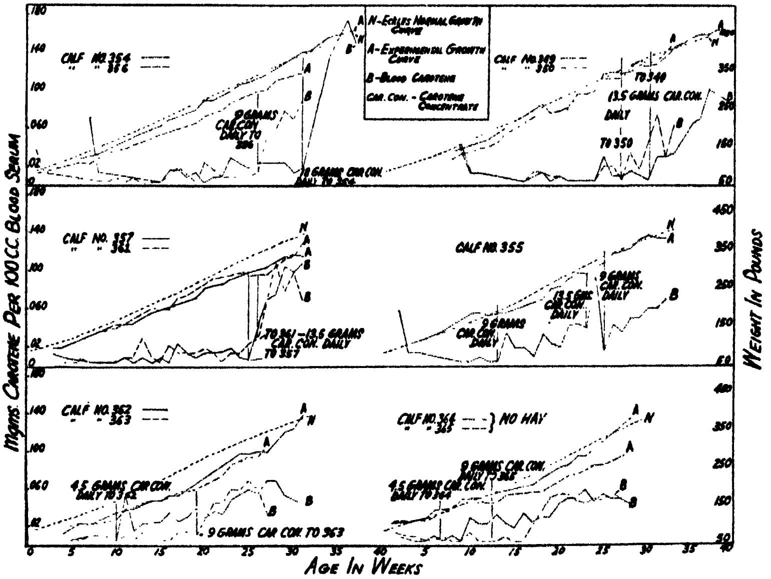

FIG. 2. Growth and blood carotene curves of calves used in second experiment.

RESULTS AND DISCUSSION

In every case following the administration of the carotene concentrate there was a marked improvement in general condition of the calf which also was usually accompanied by an accelerated growth rate. The effect of carotene administration on blood carotene varied from slight increases in some cases to very decided increases in other cases (Figure 2). The increases observed correspond roughly with the quantity of carotene administered. The relative amounts of vitamin A in the liver tissues also were found to correspond closely with the quantity of carotene supplement administered. This fact offers further proof that the carotene supplement was being utilized by the calves as a source of vitamin A.

The calves fed on the high cottonseed meal ration (calves 349, 350, 355, 364, and 365) exhibited symptoms which corresponded closely with the symptoms exhibited by those on the low vitamin A ration without cottonseed meal. They responded to the administration of the carotene concentrate in the usual manner. The calves on this ration showed a greater tendency toward intestinal disturbances and they also exhibited symptoms of vitamin A deficiency in a much shorter time than did similar calves receiving the other low vitamin A ration which contained no cottonseed meal.

The poor response of calf No. 365 while receiving no hay indicates the importance of hay as a source of carotene in the nutrition of calves. In this case distinct symptoms of malnutrition were produced very readily in the absence of both hay and carotene from its ration. Following the administration of 9 grams of the carotene supplement daily this animal showed marked improvement but never became normal. On the other hand, four and one-half grams of the carotene supplement when added to the ration of calf No. 364, was sufficient to prevent malnutrition in this animal for a considerable period (Figure 2).

Blindness was not observed in those calves in which severe avitaminosis A appeared quite rapidly. It appeared, however, in cases of those which had undergone a prolonged period of mild avitaminosis A during which the growth rate was only slightly subnormal. By carefully examining the eyes of the animals, it was found that the pupils were always dilated, and that the onset of blindness could be detected.

The carotene supplement used in this experiment, when administered in capsules and used in combination with a ration which was adequate in other respects, appeared to give results which compare very favorably with the results that had been obtained previously with alfalfa, carrots, and cod liver oil. The more clear cut and significant responses obtained in the second experiment as the result of feeding vitamin A deficient calves definite amounts of the carotene concentrate are in all probability due to improvements in technic and to more extensive data obtained through the use of a larger number of animals.

SUMMARY

Avitaminosis A was produced in growing calves through the use of poor quality bleached and aged timothy hays fed in connection with a grain mixture. Fresh carrots and alfalfa hay (dehydrated) of good quality were quite effective in correcting this avitaminosis A in calves. A commercial carotene concentrate also proved effective in preventing and curing the avitaminosis A that resulted from feeding calves rations of low carotene content, as well as overcoming the malnutrition resulting from feeding high percentages of cottonseed meal.

ACKNOWLEDGMENT

The carotene preparation used in the experiments was supplied by the Ralston Purina Company, St. Louis, Missouri.

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THE REPRODUCTIVE EFFICIENCY OF DAIRY CATTLE*

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High reproductive efficiency in livestock is economically a very desirable characteristic. The frequency of calving determines to a great extent the amount of milk a dairy cow produces during her lifetime. A review of literature reveals different methods of measuring the reproductive powers of dairy cattle.

Williams (1) recognized the economic and scientific importance of the application of some intelligible standard of breeding efficiency. He adopted two years as the ideal age at which a heifer should calve, which necessitates conception at fifteen months. Every calendar month after the fifteenth was designated as a "breeding month." Twelve months was considered as an ideal calving interval. He determined the average number of breeding months required to produce a calf by dividing the total breeding months by the number of calves born. The percentage reproductive efficiency was obtained by dividing twelve, the ideal number of breeding months for the production of a calf, by the determined average number of breeding months per calf. Williams (2) reported on a Guernsey herd which produced a calf for each 28.7 breeding months, or 41.8 per cent of ideal efficiency.

Käb (3) studied the breeding records of 1475 cows, 136 bulls and 7104 calves of the Yellow Franconian breed of dairy cattle. "An analysis of the fertility of the daughters of 22 bulls revealed considerable variation between the various families which indicated a genetic basis. Through several generations 35 families showed high fertility and 11 families low fertility."

The number of services per pregnancy and percentage of conceptions are frequently employed when measuring the breeding ability of dairy cattle (4) (5). These measures do not take into consideration the fact that many cows fail to show oestrus regularly, thereby lowering their reproductive efficiency, but not necessarily affecting the services per pregnancy. In such a calculation heifers that never calve are naturally omitted from the records. A mediocre individual might be disposed of as sterile after three or four services, whereas a more valuable animal might be given more consideration and conceive to a later service.

PLAN OF STUDY AND PROCEDURE

The study to be reported in this paper was undertaken to determine the reproductive efficiency of groups of dairy cows descended from different foundation cows. The data available for the study consist of the individual

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breeding records of 368 cows in the dairy herd owned by the Oregon State Agricultural College. Four of the major breeds of dairy cattle are represented. The records cover the period beginning with the purchase of the foundation cows in 1913 and up to 1938.

The management policies have been substantially uniform for all groups of cows. All the dairy cattle were housed and fed in the same barn, with no attempt to segregate the various breeds or groups.

Undoubtedly the most important disease factor which could affect a problem of this kind is Bang's disease. The Veterinary Department of the College began testing the College dairy herd for Bang's disease in 1919, and has continued to test at regular intervals since that time. In the fall of 1922, all of the animals which reacted to the agglutination test for Bang's disease were removed from the College dairy herd. Since that time there have been occasional removals of suspect or reactor animals. It is unlikely that any one group or breed of dairy cows intermingling freely in a herd would be more susceptible than others to an infectious disease affecting reproductive efficiency. An analysis of the groups and breeds of cows in the herd being studied shows this to be true.

The term reproductive efficiency is proposed as a measure of the net biological accomplishment of all reproductive activity. It represents the integrated effect of all the factors concerned, *i.e.*, oestrus, ovulation, fertilization, implantation, gestation, and parturition.

The numerical expression of reproductive efficiency is based on the assumption that to be 100 per cent efficient, a heifer should be bred at a certain age depending upon the breed, and that she should drop a calf every twelve months thereafter. The total number of reproductive months represent the number of months that an animal remained in the breeding herd.

As 12 months is assumed to be the desired calving interval, it follows that each cow should be credited with 12, 100 per cent months per pregnancy. Thus for convenience in obtaining a numerical expression of reproductive efficiency and for want of a better term, each month of the calving interval has been designated as one 100 per cent month. That portion of the calving interval in excess of 12 months is considered as possessing 0 per cent reproductive efficiency. Any cow known to be pregnant which was removed from the herd before calving was allowed one 100 per cent month for each month of pregnancy. *The number of 100 per cent months divided by the total number of reproductive months gives the per cent reproductive efficiency.* In the measure of reproductive efficiency proposed by Williams (1) incomplete reproductive cycles were not evaluated and heifers that never calved were not considered.

For the purpose of this study each individual foundation cow and her female descendants retained in the College herd composed a cow group. Each breed was divided into its component cow groups. The groups include from four to eleven generations and contained from 11 to 62 cows with breeding records. Heifers sold for reasons other than sterility or difficult breeding before the completion of at least one pregnancy were not included in this study.

The per cent reproductive efficiency was determined for each cow. The mean per cent reproductive efficiency with its probable error was determined for each cow group having 11 or more individuals with breeding records. Bessel's formula (6) for the application of probability to small samples was used. To determine if the differences between the various cow groups and breeds were significant, the odds against such a difference occurring due to chance were calculated. Odds of 30 : 1 or greater were considered significant.

RESULTS OF STUDY

Reproductive Efficiency of Cow Groups

In breed No. 1 foundation cows A_2, A_3, A_4 were daughters of A. In breed No. 2, foundation cow E_1 was a daughter of E, and in breed No. 3 foundation cow H_1 was a daughter of H. Since, all these cows were purchased as foundation animals the female descendants of each were considered as composing a cow group. This treatment results in duplication in the above indicated groups.

Breed No. 1. As shown in Table 1, breed No. 1 contains seven cow groups

TABLE 1

Reproductive efficiency of cow groups

Cow group	Females with herd number	Cows with breeding record	Reproductive months	100 per cent months	Mean reproductive efficiency
	No.	No.	No.	No.	%
Breed No. 1					
A	97	62	3264	2463	67.24 ± 2.51
A_2	38	25	1241	926	63.74 ± 4.68
A_3	38	22	1138	889	71.33 ± 3.55
A_4	18	12	619	468	70.57 ± 5.60
B	15	11	776	672	85.48 ± 2.44
C	13	12	709	396	54.46 ± 4.83
D	16	14	863	624	70.02 ± 4.85
Breed No. 2					
E	58	39	2032	1576	79.35 ± 1.29
E_1	49	31	2104	1519	81.86 ± 1.88
F	50	37	1574	1252	79.24 ± 2.15
G	20	14	753	571	68.83 ± 5.28
Breed No. 3					
H	71	40	2419	2037	81.90 ± 1.92
H_1	32	19	1098	999	87.22 ± 1.62
I	94	52	2851	2428	84.38 ± 1.19
J	30	16	1003	835	79.72 ± 4.46
K	19	13	653	469	68.41 ± 5.10
Breed No. 4					
L	21	11	608	477	81.97 ± 1.06

of 11 to 62 animals with breeding records. The mean per cent reproductive efficiency for the various cow groups ranged from 54.46 ± 4.83 to 85.48 ± 2.44, a difference of 31.02 ± 5.4. The odds against such a difference occurring due to chance are 6249 : 1. The foundation cows of groups A_2, A_3, A_4 were full sisters. The foundation cows of groups B, C and D were not related.

Breed No. 2. Four cow groups in Breed No. 2 contain from 14 to 39 animals with breeding records. Group E_1 has the highest mean reproductive efficiency with 81.86 ± 1.88. Group G has the lowest with 68.83 ± 5.28. The difference of 14.03 ± 5.6 with odds of 9 : 1 may not be significant.

Breed No. 3. The number of cows with breeding records in five of the cow groups of Breed No. 3 ranged from 13 to 52. The difference in reproductive efficiency between the high group H_1 and the low group K is 18.81 ± 5.35. The odds against such a difference occurring due to chance are 54 : 1.

Breed No. 4. Breed No. 4 contains one cow group of 11 or more cows with breeding records. Group L with 11 cows has a mean reproductive efficiency of 81.97 ± 1.06 per cent.

Composite Reproductive Efficiency

It will be recalled that in studying the cow groups only those groups with 11 or more cows with breeding records were considered. However, in compiling the data presented in Tables 2 and 3 dealing with the reproductive efficiency of the entire herd, all cows with reproduction records were included. This difference in procedure explains what might appear to be a discrepancy in the number of animals considered in Table 1, as compared to the numbers in Tables 2 and 3. Duplication of cows are also eliminated in Tables 2 and 3.

TABLE 2

Reproductive efficiency of herd

Breed	Females with herd number	Cows with breeding record	Reproductive months	100 per cent months	Mean reproductive efficiency
	No.	No.	No.	No.	%
1	143	99	5612	4156	67.55 ± 1.88
2	153	106	5549	4215	75.44 ± 1.47
3	214	121	6926	5769	81.27 ± 1.67
4	71	42	2269	1648	72.77 ± 1.51

Breed No. 1 has the lowest mean per cent reproductive efficiency with 67.55 ± 1.88, and breed No. 3 the highest with 81.27 ± 1.67. The difference of 13.72 ± 2.51 appears significant, with the odds of 3571 : 1 against such a difference occurring due to chance. This difference must, however, be taken to apply to the particular herd studied, rather than to breeds at large.

Table 3 shows the manner in which the individual cows were distributed with respect to reproductive efficiency.

TABLE 3

Distribution of reproductive efficiency of all cows studied

Class rep. effi.	0 %	1–20%	21–40%	41–60%	61–80%	81–100%
Breed	% of all animals of each breed					
1	9.6	0	8.6	12.4	34.6	34.5
2	3.6	0	0.9	10.9	36.6	47.6
3	2.3	0	0	8.5	27.0	61.9
4	0	0	2.3	23.2	34.8	39.5
Herd	4.42	0	2.85	11.95	32.73	48.05

This treatment of the results strengthens the contention that the cow groups in this study possess significant differences in reproductive efficiency.

DISCUSSION

It is realized that in a study of this type some assumptions are necessary, and it is impossible to eliminate all factors that may affect the results obtained. Apart from the procedure and numerical method of expressing reproductive efficiency two questions arise which have important bearing on the validity of the conclusions arrived at.

One question is the number of animals necessary for such a study. In comparing cow groups within the breeds only those with 11 or more cows with individual breeding records have been considered. Inasmuch as this study involves the reproductive performance of 368 animals it is felt that the results should possess a fair degree of reliability.

One of the most difficult problems in a study of this sort is to assess the influence of the herd sire. It is not to be inferred that the impaired reproductive efficiency encountered in this analysis was not in part due to the sires. Since, however, this study extended over a period of 24 years and involved some 21 bulls for breed No. 1, 17 bulls for breed No. 2, 10 bulls for breed No. 3, and 16 bulls for breed No. 4, obtained from widely different sources and largely unrelated, it does not appear likely that the influence of any sires of low fertility was concentrated in any one breed or group. This is strengthened by the fact that widely different efficiencies were obtained with substantially the same sires. For example, in breed No. 1, cow group C shows a very low mean reproductive efficiency, while cow group B shows a very high mean reproductive efficiency.

Another method of approach which indicates that the difference in reproductive efficiency between cow groups is significant, deals with the correlation between the reproductive efficiency of the foundation cows of the groups studied and the mean reproductive efficiency of their female descendants. The value obtained for this correlation was $r = +.546 \pm .118$. Although the number of animals considered in this correlation is limited it appears that the importance of selecting foundation cows with high reproductive efficiency should be strongly emphasized.

SUMMARY

1. A numerical method of measuring the reproductive efficiency of dairy cattle has been suggested.

2. This measure has been applied to the breeding records of the herd of dairy cows owned by the Oregon State Agricultural College during a 24 year period.

3. In the herd studied a marked difference in reproductive efficiency between various cow groups and between breeds has been noted.

4. A correlation of r = +.546 ± .118 was found to exist between the reproductive efficiency of the foundation cows and the mean reproductive efficiency of their respective female descendants.

5. The study suggests that the reproductive efficiency of the foundation cow is an important factor in determining the mean reproductive efficiency of her offspring.

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A NOTE ON THE SUITABILITY OF VARIOUS LOTS OF DYE FOR USE IN THE RESAZURIN REDUCTION TEST[1]

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Most of the studies reported in the literature on the use of the resazurin reduction test for grading milk and other fluid dairy products seem to have been made using dye obtained from one manufacturer. Although other brands have been available, they apparently have not been used by dairy sanitarians in this country. When using one of these other brands of dye, the author obtained results less satisfactory than those reported in the literature. This led to comparisons of four different brands of resazurin. The brands were designated A, B, C and D, and each was obtained in the original package of the manufacturer whose label it bore. The comparative tests were made using Ramsdell's procedure (1) on samples of aseptically drawn and market milks. Some of the tests were made within an hour or two after the samples had been taken, the samples being held in ice during the interim. The other tests were made on samples which had been held overnight at 3–5° C. All tests were observed at hourly intervals for at least five hours. The colors of the dye in acid and alkaline solution were obtained by adding excesses of dilute hydrochloric acid and sodium hydroxide, respectively, to the stock solutions.

The results of the comparisons of the different brands of dye are shown in Table 1. Brand A is the one which has been used in most studies of the resazurin test. The lot of this dye which was used underwent the "normal" sequence of color changes and was used as a standard of comparison. Brand D also gave satisfactory results in the test. In the two instances in which results with brand D were not identical with those obtained using brand A, the more rapid color change undergone by brand D was slight but of sufficient magnitude to be observed easily. Brands B and C were entirely unsuited for use in the resazurin reduction test, the only change in color, that to the completely reduced form, occurring too slowly to be of practical use.

Of the four brands of resazurin examined, only two, A and D, were satisfactory for use in the resazurin reduction test as applied to milk. The exact nature of the differences between the four lots of dye was not determined. If the resazurin test is to be adopted generally, the need of standardization of the dye and of certification of different lots of dye by a responsible agency is definitely indicated.

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TABLE 1

Comparisons of characteristics of different brands of resazurin for use in the resazurin reduction test

Brand	Solution characteristics					Results of use in reduction test	
	Ease of solution*	pH of stock solution	Color of stock solution	Color in acid solution	Color in alkaline solution	Color change in milk	Results obtained
A	Easily soluble	7.55	Purplish blue	Pinkish rose changing to moderate orange	Clear blue	Blue→lavenders →pink→colorless	Satisfactory
B	Slowly and incompletely soluble	....	Muddy blue	Muddy blue	Muddy blue	Muddy blue→colorless	Unsatisfactory. Color change occurs at about time of pink→ colorless change of brand A
C	Almost insoluble in water. Incompletely soluble after addition of alkali to pH 7.88	7.88	Purplish rose, resembling colloidal gold sol.	Moderate orange	Clear blue	Pink→colorless	Unsatisfactory. Color change parallels last change of brand A
D	Easily soluble	7.60	Purplish blue	Pinkish rose changing to moderate orange	Clear blue	Blue→lavenders →pink→colorless	Satisfactory. Same results as with brand A in all but 2 of 102 comparisons

* 0.05% dye in distilled water.

THE RELATIONSHIP BETWEEN FAT CONTENT OF FRESH MILK AND ITS APPARENT ACIDITY

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For a great number of years the titratable acidity test for acid in milk has been used as a means of measuring the quality of milk for condensing, evaporating, drying, and for other general uses. It has long been known by scientists and creamerymen that fresh raw milk which contains no lactic acid will test from .07 to .28 per cent "lactic" acid as measured by this test. This test was first adopted because of the fact that high-acid milk generally has a low heat coagulation point. The revelations of more recent investigations (1) have shown that the titratable acidity is not the only, and in many instances, not the dominating factor determining the behavior of milk toward the manufacturing process.

It has also been shown that the apparent acidity has no effect on the heat coagulation point of milk (2) and should, therefore, have no part in the determination of the fitness of milk for condensery purposes, that the natural acidity constitutes a large part of the titratable acidity in milk when it is delivered to the processor and may in some rare instances even be above the limit of acidity set for acceptable milk, (3) and that, therefore, the titratable acidity is not an accurate index to the fitness of milk for manufacturing (4).

Due to the many shortcomings of the titratable acidity test, scientists have set forth in an earnest endeavor to discover or devise a more suitable test for measuring the true fitness of milk for manufacturing. The pH test for hydrogen-ion concentration in milk has been suggested, the alcohol test has been used to a considerable extent, the phosphatase test has been used as a supplementary test and many other tests have been used. These tests are all deficient, however, in requiring more skill in operation, more equipment, and more time and their value is questionable in many instances.

L. A. Rogers and his co-workers, (1) in their study of the relation of acidity to the coagulation temperature of evaporated milk, say that notwithstanding the generally accepted views as to the usefulness of grading milk on the basis of titratable acidity, the results obtained by determining the hydrogen-ion concentration electrometrically do not indicate that the relation between the acidity of fresh milk and the coagulating point after evaporation is sufficiently uniform to be of much value in grading milk. This is evident, they further say, because the hydrogen-ion concentration that gives maximum stability to the casein varies with the composition of the milk.

On the basis of their findings, they conclude that there is no very definite relation between the coagulating temperature of evaporated milk and the

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true acidity, as measured by determining the hydrogen-ion concentration of the milk before sterilization, and a very small increase in the acidity from the normal for that milk will cause a distinct lowering of the coagulating temperature of the evaporated milk.

Fleming and Nair (5) after an extensive study of the value of the titration test for acidity, made the following conclusions: the normal acidity of composite herd milk as received at the factory is 0.140 per cent; that where milk is handled in a clean and careful manner it will not be rejected on the basis of any acid test now in use; the value of the acid test in checking the methods of production is, accordingly, clearly demonstrated and its ease of application combined with this demonstrated reliability warrants its use until such time as some other equally rapid and more dependable method of determining the quality of milk has been perfected; that the percentage of cows and herds showing abnormally high acid is so small as to be negligible and that the manufacturer is doing no injustice to the producer when he insists that all milk must be delivered with an acidity below 0.17 or 0.18 per cent, particularly when such test is applied as an adjunct to the nose and palate of the experienced inspector.

Sommer and Hart (6) suggest the following procedure in cases where the acidity is persistently high and very evidently due to a high apparent acidity.

1. Determine the apparent acidity of the milk at intervals of several weeks.
2. Allow an increase in acidity (*e.g.*, 0.03 per cent) due to slight fermentation that is likely to occur before delivery.
3. If the apparent acidity at the farm was 0.17 per cent, then reject the milk if the acidity exceeds 0.20 per cent.

McInerney (7) concludes after an investigation on the acidity of fresh milk that fresh milk which shows a high percentage of solids not fat and a high percentage of ash shows a high percentage of apparent acidity and that fresh milk comparatively low in solids not fat and in ash shows a lower percentage of apparent acidity.

Rice and Markley (8) studied the natural acidity in freshly drawn milk and accounted for it as follows:

CO_2	in	amount	equivalent	to	.01	to	.02%	lactic	acid
Citric acid	"	"	"	"	.01	"	"	"	"
Albumin	"	"	"	"	.01	"	"	"	"
Casein	"	"	"	"	.05	"	.08%	"	"
Phosphates	"	"	"	"	the	difference			

It is well known that in milk having a high fat content there is also a high percentage of ash and solids-not-fat, such as casein and albumin. On the basis of the conclusions of Fleming and Nair, and of McInerney, and the suggestions of Sommer and Hart, and the findings of Rice and Markley, this investigation was undertaken. To the author it seemed logical to conclude that since the fat generally varies directly as the solids not fat, though not proportionately, and since milk high in solids not fat is also high in acidity, there should also be some relationship between fat content of milk and titratable acidity.

EXPERIMENTAL

Samples of milk from individual cows in the University herd were taken each milking for a period of about 4 months. The University herd is made up of cows of the Holstein, Jersey, and Guernsey breeds. As soon as a sample was taken, a 9 c.c. portion was pipetted into an evaporating dish and about 5 drops of a 1 per cent alcoholic solution of phenolphthalein indicator added. N/10 normal NaOH was then stirred into the mixture until it became a faint pink and remained so upon continued stirring. Care was taken not to exceed the end point and all samples were tested as uniformly as possible. The remainder of the sample was then tested for butterfat by the regular Babcock procedure. Any abnormalities in the condition of the cow were carefully noted. Approximately 1300 samples were run in all and the experiment was continued from February into July. These months represent nearly a maximum variation in weather conditions in Arizona. A biometrical study was then made of the data on hand, the results of which are presented herein.

RESULTS

The results of this study showed a constant increase in the titratable acidity of milk as the per cent of fat in the milk increased up to five and nine tenths per cent. Above this point the acidity decreased. This was thought to be due to the fact that milk containing above 5.9 per cent fat is unusual and consequently often abnormal; abnormal milk usually tests low in apparent acidity. For an increase of 4 per cent in fat there was an increase of .02328 per cent in apparent acidity. This is equal to .00582 per cent average increase in apparent acidity for every one per cent fat increase.

TABLE 1

Mean titratable acidity for different levels of fat

Fat %	Mean	Standard deviation
2.0–2.4	.10967	.01804
2.5–2.9	.11457	.01458
3.0–3.4	.12129	.01571
3.5–3.9	.12215	.01216
4.0–4.4	.12651	.01641
4.5–4.9	.12825	.01533
5.0–5.4	.13237	.01486
5.5–5.9	.13495	.01545
6.0–6.9	.12700	.01454
7.0 and above	.12269	.01342

Difference 2.0 to 5.9% fat = .02328 per cent apparent acidity.
1267 samples

Actually, in some instances the increase was slightly greater than this and in some cases it was a little less. (See Table 1.)

It was shown that the normal variation in the acidity of milk of different levels of fat from the Holstein and Guernsey breeds was approximately the same as when all milk was considered together. In Jersey milk this was not found to be the case. The variation was very small in Jersey milk. (See Table 2) This can probably be partially accounted for by the fact that the normal variation in fat content of milks from different cows of the Jersey breed is less than is the case in the Guernsey and Holstein breeds, thus the range of normal milk in the case of the Jersey is less than with the Holstein and Guernsey.

TABLE 2

Per cent acidity at different levels of fat in milks from different breeds

Jerseys			Guernseys			Holsteins		
% Fat	Mean	Standard deviation	% Fat	Mean	Standard deviation	% Fat	Mean	Standard deviation
						Below 3.0	.11287	.01562
			Below 4.0	.12381	.01362	3.0–3.9	.12199	.01396
Below 5.0	.12829	.01437	4.0–4.9	.12955	.01404	4.0–4.9	.12671	.01570
5.0–5.9	.13169	.01614	5.0–5.9	.13516	.01367	5.0 and above	.12615	.01389
6.0–6.9	.13196	.01361	6.0 and above	.12071	.01193			
7.0 and above	.12421	.0370						

A study of the titratable acidity at different levels of fat from individual cows shows that as a general rule the titratable acidity of the milk from a single cow tends to remain fairly constant and does not vary directly or indirectly with the fat. This is to be expected as it has often been shown that the solids not fat in a cow's milk tends to remain fairly constant under normal, constant, environmental conditions (13).

It was found that as the weather warmed up, the mean titratable acidity of all the milk tested tended to go down while the fat remained fairly constant. (See Table 3.) This can be expected because several experiments

TABLE 3

Monthly variation in per cent acidity and fat

Month	% fat	Mean titratable acidity
March	4.309	.13045
April	4.350	.12567
May	4.293	.12226

have shown that as winter conditions change to summer, the solids not fat content of the milk produced tends to decrease. (9, 10, 11.) As the S.N.F. decreases, the acidity would also decrease (8).

As is shown in Table 4, in the morning the increase in titratable acidity for each increase of one per cent of fat was not as great as in the afternoon milking. In the afternoon, the milk varied slightly more than the average increase in acidity for the milk taken as a whole. In the morning, the opposite was the case.

TABLE 4

Variations in per cent acidity at various levels of fat from morning to night

Morning		Afternoon	
% Fat	Mean titratable acidity	% Fat	Mean titratable acidity
Below 3.0	.11789	Below 3.0	.10931
3.0–3.9	.12385	3.0–3.9	.12087
4.0–4.9	.12607	4.0–4.9	.12786
5.0–5.9	.12818	5.0–5.9	.13426
Above 6.0	.11864	Above 6.0	.12778

The acidity of the milk from cows that were sick was found to be consistently abnormally low. (See Table 5.) This was not due to udder infection in these cases, but the cows were sick from digestive disturbances.

TABLE 5

Effect of sickness and injury, etc.

Per cent acidity	Fat
.11	4.6
.10	7.3
.12	6.8
.10	5.4
.09	4.4

Three cows in the herd showed figures which were abnormal throughout the test period. Two of the cows had milk which tested abnormally low in acidity at all times, while the other animals' milk tested abnormally high in acidity at all times. (See Table 6.) These abnormal cows are often found in herds, but do not ordinarily influence materially the mixed herd milk.

Cows near the end of their lactation period were found to produce milk which was abnormally low in acidity. (See Table 7.) This is contrary to the findings of McInerney (7) but confirm the reports of Miyawaki (12).

It was found that the milk from fresh cows is abnormally high in acidity, but that as time progresses the milk goes lower and lower in acidity until after approximately 30 days the milk is normal. (See Table 8.) This is

TABLE 6

Abnormal cows

Ruby		No. 193		No. 199	
Per cent acidity	Fat	Per cent acidity	Fat	Per cent acidity	Fat
.12	4.7	.09	2.7	.15	4.4
.11	6.7	.10	2.8	.15	4.8
.12	4.0	.10	2.6	.15	4.0
.13	6.2	.10	2.6	.15	3.9
.13	4.5	.10	2.8	.14	4.2
.11	6.6	.08	3.2	.15	4.9
.12	4.5	.07	3.4	.15	5.4
.12	6.0	.08	3.3	.15	5.4
.12	6.5	.08	2.8	.16	4.1
.10	3.9	.07	1.9	.15	5.0
.11	6.4	.10	2.7	.15	4.6
.12	4.8	.09	3.0	.14	4.5
.10	6.4	.10	2.7	.15	4.5
.12	5.1	.10	2.9	.14	4.6

also contrary to the observation of McInerney (7) but again confirms the work of Miyawaki (12).

TABLE 7

Percent of acidity in milk of cows near end of lactation

No. 83		No. 95	
Per cent acidity	Fat	Per cent acidity	Fat
.11	4.5 3/15/38	.12	8.5 5/17/38
.11	6.1	.13	6.0
.11	6.3	.12	8.7
.09	5.7	.13	6.9
.09	4.7	.12	6.0
.10	7.2	.12	5.6
.08	5.2 dried up 4/14/38	.11	9.3 dried up about 6/1/38

SUMMARY AND CONCLUSIONS

Data are presented herein which show that milk in general increases in apparent acidity as the fat content increases up to 6 per cent butterfat, from this point on the acidity decreases. This phenomena was also noted in milk taken from Holstein cows exclusively and from Guernsey cows exclusively, but it was not so apparent in Jersey milk. Individual cows likewise did not show this variation. As summer conditions became prevalent, the mean titratable acidity of milk in general decreased. Milk from the morning milking did not display the same trend in variation in acidity that was displayed by milk from the afternoon milking.

A small number of cows tested showed abnormal tests at all times. Two animals producing milk which was always abnormally low in acidity and one cow produced milk that was always abnormally high in acidity.

Cows advancing in their lactation period and sick cows produced milk

TABLE 8

Per cent acidity in milk of fresh cows

Royal Belle		201		Pansy		87		209	
% Acidity	Fat	% Acidity	Fat	% Acidity	Fat	% Acidity	Fat	% Acidity	Fat
3/16/38 1st Milking		3/21/38 1st Milking		3/25/38 1st Milking		Fresh 3/28/38		3/3/38 1st Milking	
.17	5.6	.23	4.7	.11	6.2	.15	2.9	.14	4.3
.17	5.2	.20	6.5	.12	2.6	.15	4.3	.14	4.1
.16	5.1	.19	4.4	.15	5.8	.15	4.2	.14	4.7
.16	5.5	.17	4.7	.15	5.3	.15	4.7	.14	3.3
.17	4.2	.16	3.8	.15	5.4	.14	6.7	.12	4.1
.15	4.4	.17	3.6	.14	3.4	.14	4.9	.12	4.2
.16	4.0	.16	3.1	.15	3.9	.15	6.0	.12	4.6
.15	4.5	.15	4.5	.14	5.6	.13	5.7	.13	3.7
.16	4.1	.15	3.5	.15	4.1	.14	5.4	.13	3.3
.15	4.5	.15	4.0	.15	5.1	.13	5.7	.13	4.6
.16	4.7	.13	3.3	.14	5.9	.12	5.3	.13	3.4
.15	5.3	.13	4.5			.14	5.5	.13	3.9
.15	5.0	.13	4.4			.12	5.7	.13	3.5
.14	5.0	.12	4.1			.13	6.3	.13	4.2
.14	5.0	.14	3.2			.12	5.4	.13	2.9
.15	4.5	.13	3.5			.12	6.0	.13	3.4
.15	4.9	.12	4.8			.12	6.0	.13	3.3
.14	5.0	.12	3.5			.12	5.3		
		.12	4.2						
4/27/38		4/4/38				4/20/38			

abnormally low in acidity. Cows after freshening, for a period of about 30 days, produced milk abnormally high in acidity.

On the basis of this study the author is inclined to agree with the conclusions of Fleming and Nair (5) namely that the ease of application of the acidity test and its reliability warrant its continued use until some better test is found and that it does not normally work an injustice on the producers of even the richest milk. The author urges, however, that users of the titratable acidity test adopt a limit of acidity at which milk will be acceptable that varies directly as the fat, increasing the limit .01 per cent for every 2 per cent increase in fat content up to 6 per cent. For example, a standard for 2 per cent milk could be set at .17 per cent acidity, for 3 per cent milk at .175 per cent acidity, .18 per cent for 4 per cent milk, and .19 per cent for 6 per cent milk, or any other standard which they might choose as being suitable for their conditions.

The recommendations of Sommer and Hart (6) are also heartily approved of as being worth-while steps to take in the case of consistently high acid milk.

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OXIDIZED FLAVOR IN MILK. VII. STUDIES OF THE EFFECT OF CAROTENE AND ASCORBIC ACID IN THE FEED OF THE COW ON THE SUSCEPTIBILITY OF THE MILK TO METAL-INDUCED OXIDIZED FLAVOR

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Feed was one of the earliest factors known to influence the susceptibility of milk to oxidized flavor. Kende (10) in 1932 reported that oleaginous (oxidized) flavor in milk seemed to occur only during the winter, and that green feed as well as fresh hay produced from it contained considerable amounts of reducing substances which tended to prevent oxidized flavor from developing. Guthrie and Brueckner (9) concluded that dry feed was not the sole cause of the development of this defect inasmuch as there was a variation in the intensities of the oxidized flavors from different quarters of the same udder. Anderson (1) and co-workers (2) have shown that feeding carrots or artificially dried alfalfa aided in eliminating this defect. Their observations led them to believe that off-flavored milk from individual cows might be caused by dietary deficiencies.

Whitnah, Martin, and Beck (14) have found that all samples which develop oxidized flavor are below the breed average in intensity of fat color. However, not all samples low in color develop an off-flavor. In the feeding of dehydrated young oat plants (containing 103 mg. of carotene per lb. of plants) and carotene concentrate (containing 150 mg. of carotene per lb. of concentrate) it was found that 1 pound of the concentrate was equivalent to about 4 pounds of the dehydrated oat plants in improving the flavor. This difference could not be accounted for on the basis of carotene content alone. They eliminated this flavor defect in two to four days by feeding the concentrate. The color of the fat was not increased for some time after the flavor improved. Whitnah *et al.* (15) found that supplementing the ration with carotene produced an increase in the carotene content of the blood and eliminated the oxidized flavor in the milk of individual cows. Stebnitz and Sommer (12) found no relation between the carotene content, as evidenced by the color of the fat and the stability of the fat toward oxidation.

Garrett *et al.* (7) (8) found a correlation of 0.6 between vitamin C content and the flavor of the milk from individual cows. When the vitamin C content of the milk was high the flavor of the milk was usually good. Also

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a correlation was found between the color of the milk and the flavor. They concluded that a definite relationship exists between the vitamin C content and the yellow color in the milk from individual cows of both the Guernsey and Holstein breeds as indicated by correlation coefficients of over 0.5 and over 0.6, respectively.

Dahle and Palmer (6) were unable to eliminate oxidized flavor by the direct addition of 8 mg. of pure beta-carotene per liter of milk.

Chilson (5) substantiated the work of Kende (10) that the direct addition of small quantities of ascorbic acid to milk would prevent the development of oxidized flavor.

More recently Brown (4) and co-workers have demonstrated that the feeding of lemon juice and tomato juice to cows on dry feed greatly reduced the susceptibility of the milks to oxidized flavor development. They explained this effect on the basis of the vitamin C contained therein, since they found that the daily feeding of 0.5 gram of ascorbic acid reduced the susceptibility.

Because it is now possible to supplement the basic ration of the cow with either ascorbic acid or carotene from commercial sources, it seemed desirable to know whether carotene or ascorbic acid, or both, were necessary in order to eliminate the susceptibility of the milk to oxidized flavor. Although carotene has been shown to eliminate naturally occurring oxidized flavor in the milk of individual cows, no studies have been reported on the effect of a carotene supplement on metal-induced oxidized flavor. Likewise, further study is needed concerning the manner in which ascorbic acid affects the susceptibility of the milk to the development of oxidized flavor. To obtain further information on these points the following experiment was planned and conducted.

EXPERIMENTAL

For use in this experiment, six cows, whose milks were susceptible to oxidized flavor when contaminated with copper, were selected and placed on a ration low in carotene. The ration consisted of a grain mixture of 150 lbs. ground oats, 100 lbs. cottonseed meal, 100 lbs. wheat bran, 5.3 lbs. salt, and 7 lbs. of steamed bone meal, with timothy hay as the roughage.

Samples of milk were collected from each cow each evening and pasteurized in bottles. Following pasteurization the samples were divided into two lots and one lot was contaminated with copper at the rate of 1.3 p.p.m., added from a copper sulphate solution, while the other served as a control. Both samples were then stored in ice water for three days, after which they were scored for flavor by at least three judges familiar with oxidized flavor. On three consecutive days each week, large samples of the milk from each cow were taken and vitamin C and carotene determined. The vitamin was determined on individual samples by titration as suggested by Sharpe (13). The samples of milk were prepared for carotene analysis by gravity separation of the cream followed by churning in a small glass churn. The butter thus obtained was melted and centrifugalized in Hart's casein tubes in an electrically heated centrifuge for 15 minutes, after which the clear liquid butter oil was decanted into a clean dry jar. A composite sample was made from this butter oil of three consecutive days and the carotene analysis made according to the method of Baumann and Steenbock (3) as modified by Rogers and associates (11).

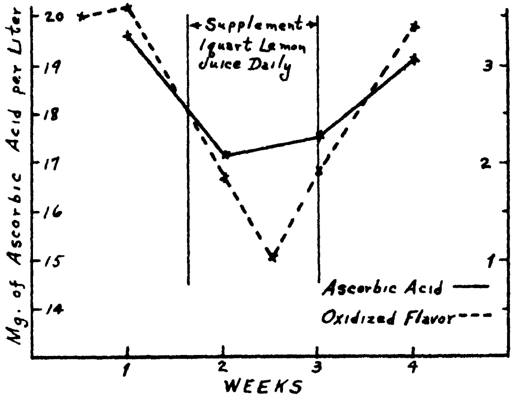

FIG. 1. The Effect of Lemon Juice Supplement on the Ascorbic Acid Content of the Milk and the Susceptibility of the Milk to Oxidized Flavor. Oxidized flavor values: 1, very slight oxidized flavor; 2, slight oxidized flavor; 3, moderate oxidized flavor; 4, pronounced oxidized flavor.

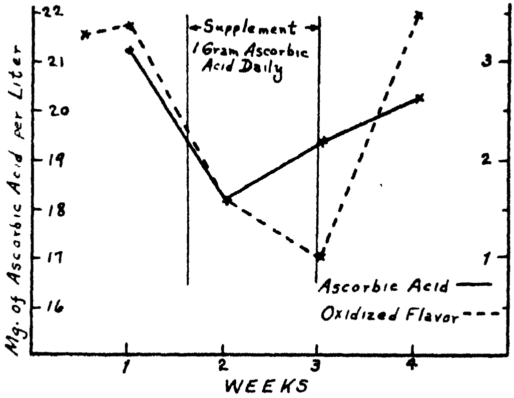

FIG. 2. The Effect of Ascorbic Acid Supplement on the Ascorbic Acid Content of the Milk and the Susceptibility of the Milk to Oxidized Flavor.

After a five-day preliminary trial on the new ration the cows were divided into two groups of three cows each. Group one was given a daily supplement of one quart of freshly extracted lemon juice while the other was given a daily supplement of one gram of crystalline ascorbic acid. Both groups were held on the basic ration with the vitamin C supplement for ten days, during which time the effect of the supplement to the low carotene ration was being studied in relation to metal-induced oxidized flavor. The results of this study are shown in figures 1 and 2. Because all control samples (not contaminated with copper) were free from oxidized flavor they were omitted from the graph.

Figure 1 shows the results obtained with lemon juice while figure 2 shows the results of the ascorbic acid supplement. Although the oxidized flavor was not entirely eliminated, it was greatly decreased as a result of the vitamin C supplement. With four of the six cows used in this experiment the flavor was decreased to the point where for three consecutive days no oxidized flavor was detectable. In the milk from the other two cows the flavor was greatly decreased but not entirely eliminated. Presumably the flavor could have been eliminated entirely had the supplement been increased or continued for a longer period.

The next ten days following the discontinuance of the vitamin C supplement, the cows were continued on the basic ration and the intensity of the oxidized flavor again reached an intensity similar to that observed at the start of the experiment. At this time a carotene supplement was added (Pur-A-Tene—carotene in oil) to the ration at the rate of ½ pound (containing 350 mg. of carotene) per cow per day for a period of 20 days. During this period the effect likewise was studied of the carotene content of the ration on the intensity of the metal-developed oxidized flavor of the milk.

The results for four of the cows used in this trial are shown in figure 3, while the data for the two remaining cows are shown in figure 4. The oxidized flavor in the milk from the cows whose data are shown in figure 4 did not decrease as rapidly as it did in the other animals and for this reason

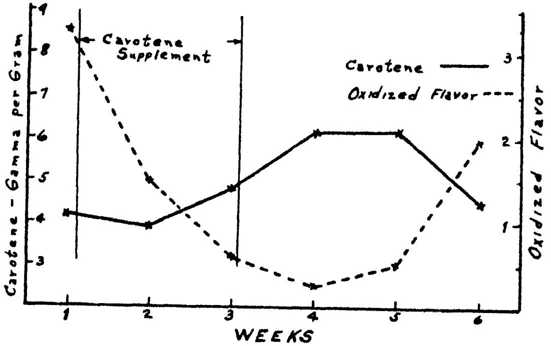

FIG. 3. The Effect of the Carotene Supplement on the Carotene Content of the Milk and the Susceptibility of the Milk to Oxidized Flavor.

the carotene supplement was doubled for an additional week. This was sufficient to reduce greatly the intensity of the oxidized flavor developed. The results of these trials show that a carotene supplement in the feed will render the milk non-susceptible to metal-induced oxidized flavor. As in the previous experiment, all the control samples were free from oxidized flavor and hence were omitted from the figures. Supplementing the ration with carotene resulted in an increase in carotene in the milk.

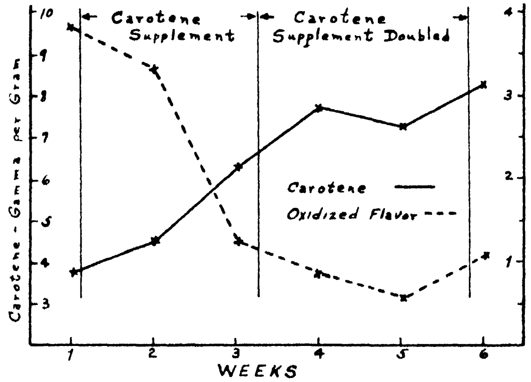

FIG. 4. The Effect of the Carotene Supplement on the Carotene Content of the Milk and the Susceptibility of the Milk to Oxidized Flavor.

DISCUSSION

The results of this experiment show that the addition of either ascorbic acid or carotene to the ration will render the milk less susceptible to metal-induced oxidized flavor. By supplementing the ration with carotene, the carotene content of the milk is increased, while the addition of ascorbic acid to the ration does not seem to increase materially the ascorbic acid content of the milk. Apparently the ascorbic acid supplement acts in some manner other than by directly increasing the ascorbic acid content of the milk. The addition of one gram of ascorbic acid or one quart of freshly extracted lemon juice to the ration of each cow resulted in a slight increase in the carotene content of the milk produced. Likewise, when carotene was added to the ration there resulted an increase in the ascorbic acid content of the milk. Despite considerable daily variation in the ascorbic acid content of the milks, when the three consecutive days were averaged, this trend is still evident. No explanation is offered at this time for this effect. A typical example of this relationship can be seen in the complete data for one cow, shown in figure 5, but all of the cows showed a similar tendency.

It was thought at the beginning of the experiment that when the animals were placed on a low carotene ration some of their milks might develop an oxidized flavor spontaneously. However, this did not occur, indicating that some factor other than a low carotene content is responsible for the incidence of naturally occurring oxidized flavor.

These results are in agreement with the findings of Anderson and his co-workers in that carotene in the ration tends to render the milk non-susceptible to oxidized flavor development. The discrepancy with the work of Stebnitz and Sommer may be explained on the basis that they were working on the ordinary chemical oxidation of butter fat when they found no relation between the carotene content, as evidenced by the color, and the stability of the fat toward oxidation, whereas the oxidation involved in the process of developing oxidized flavored milk is somewhat more involved.

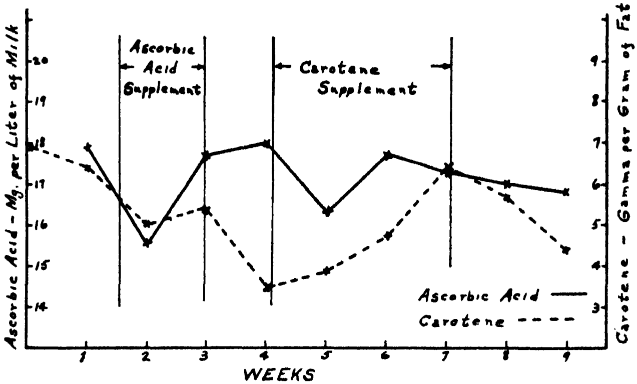

FIG. 5. Effect of Ascorbic Acid Content of Ration on Carotene Content of Milk and Effect of Carotene Content of Ration on Ascorbic Acid Content of Milk.

In the work of Dahle and Palmer the carotene was added directly to the milk and, in order to affect the susceptibility of the milk, it must apparently be in solution in the fat.

SUMMARY AND CONCLUSIONS

1. Ascorbic acid fed at the rate of one gram per day reduced considerably the tendency for metal-induced oxidized flavor to develop, although it did not materially increase the ascorbic acid content of the milk.

2. Carotene fed at the rate of 350 mg. per day greatly reduced the tendency for metal-induced oxidized flavor to develop and resulted in an increased amount of carotene in the milk.

3. The mechanism whereby oxidized flavor is developed spontaneously is influenced by some factor or factors other than that the ration is low in carotene.

4. Ascorbic acid fed to cows on a ration low in carotene seemed to increase slightly the carotene content of the milk produced, and, conversely, feeding carotene to the same animals on the low-carotene ration apparently increased somewhat the ascorbic acid content of the milk.

ACKNOWLEDGMENT

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WATER SORPTION BY DRY MILK SOLIDS. I. THE EFFECT OF PROCESSING TREATMENTS ON VOLUME CONTRACTION

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Dry milk solids occupy an important place in the dairy industry, being used for human food, animal feed and for some industrial purposes, with human consumption taking the largest amount. Among the human food uses most are affected by the dry milk solids—water relationships. For reconstituting to the fluid state complete solubility (actually colloidal dispersion) is desirable. For use in bread making, which is one of the more important human uses, complete solubility is not required but the maximum ability to hold water in a fixed state is desirable. The effects of processing treatments upon solubility have been established and are well known. The factors affecting water sorption have not been established. It is the purpose of this study to investigate certain phases of this problem.

Water sorption is the taking up of water by colloidal materials and holding it with some degree of force. It includes absorption, an intramicellar phenomenon, and adsorption, a surface or intermicellar relationship. It is considered synonymous with hydration, but may be different from the manifestation of "bound water" as commonly measured. Water sorption exhibits itself through a positive heat of reaction as contrasted to solution which is endothermic; through a lowering of the aqueous vapor pressure; through a swelling of the colloidal material showing a definite swelling pressure; and through a volume contraction of the entire system.

REVIEW OF LITERATURE

So voluminous is the material that it is beyond the limits of this paper to attempt a complete bibliography covering the subject of hydration and its measurement. Consequently only that literature will be cited which has direct bearing on the subject at hand.

There have been many methods devised for the estimation of the water sorption capacity of colloidal materials. Newton and Gortner (1) have devised a technique for measuring "bound" water that depends upon the depression of the freezing point. Briefly, the freezing point of a solution of the material is measured and then a predetermined quantity of sucrose is dissolved in the liquid and the freezing point depression noted. The difference between the theoretical depression and the actual depression is caused by some of the water being "bound," or not free to dissolve sucrose. The quantity can be calculated from the above values. Gortner and Sharp (2) have used viscosity as a measure of hydration of wheat flours; the flour with the greater hydration capacity will yield the more viscous solution.

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Hansen (3) has used the heat of hydration given off when dry milk is put into water as a value from which to calculate the moisture content of the dry milk. Obviously, such a method in order to be valuable for moisture determination must be applied to dry milks of the same capacity to become hydrated. Kuhlman and Golossowa (4) have used a modification of Newton and Gortner's "bound" water method in studying breadmaking. They have substituted the determination of the refractive index for the freezing point. Thiessen (5) has described a number of methods for measuring the swelling of colloidal materials. These include measuring the increase in weight, the aqueous vapor pressure of the material at definite water contents, and the increase in dimensions of the swelling material.

The thermodynamic relations of water sorption have been studied by various investigators. Fricke and Lüke (6) have shown that there is a direct relation between volume contraction and heat of imbibition and that casein shows an integral volume contraction of approximately 40 cubic millimeters per gram of dry material. Katz (7) has discussed the laws of swelling from the thermodynamic viewpoint. Stamm (8) has investigated the relationships between water and cellulosic materials, particularly wood, and applied thermodynamic interpretation to his results.

The dilatometer has been used for many years as an instrument with which to measure small changes in volume. Svedberg (9) used a dilatometer for studying the hydration of gelation. He found a volume contraction of 54 cubic millimeters per gram of gelatin. Hampton (10) in a brief note has described a dilatometer designed particularly for measuring the hydration of colloids. Soil scientists have frequently employed the dilatometer to determine the water holding capacity of soils. An example is given by Bouyoucos (11). Cole (12) has used a dilatometer in the field of dairy products to study freezing in ice creams. Recently, Rishoi and Sharp (13) have measured the volume change accompanying fat solidification in cream in a dilatometer.

The freezing point "bound" water technique of Newton and Gortner described above has been used in connection with dairy products. Pyenson and Dahle (14, 15, 16) have made an extensive investigation of dairy products using this method. They report that the solids of skim milk "bind" 29.1 grams of water per 100 grams of solid when rennet coagulated and 25.5 grams per 100 grams of solid when acid coagulated. Concentrated milk is reported to bind 36–39 grams of water per 100 grams of milk solids. Fresh dry milk solids (vacuum drum process) showed a water binding capacity of 46.5 grams per 100 grams solids immediately upon wetting and 61.1 grams after aging 24 hours in the solution. The water binding capacity of the dry milk solids had decreased four weeks after manufacture and still further after eight weeks. Gelatin showed no water binding capacity by this method.

Skovholt and Bailey (17, 18) have compared baking qualities of dry milk solids prepared by different methods, also they have attempted to correlate the viscosity of the water suspensions of dry milk solids with water absorption in bread dough. Roller process solids gave higher viscosities but not higher absorption in the doughs. They state that water absorption in the dough is not directly measured by viscosity of the suspension and that these properties do not parallel baking strength. Likewise, Johnson and Ward (19) have reported that the viscosity of condensed skim milk is not related to baking quality. Greenbank and associates (20) also have studied the effect of heat on the baking quality of milk. They consider the viscosity of the reconstituted product to be a measure of the water holding capacity of the dry milk.

Supplee (21) made a study of the moisture content of milk powders in equilibrium with atmospheres of varying humidities. He found that milk powders prepared in different ways varied somewhat in their ability to take up moisture from the air. His method was to suspend the powder in an atmosphere of constant known humidity and determine the moisture content at equilibrium. Samples represented powders manufactured by the roller and by the spray processes, with and without precondensing. Values are given for humidities as high as 80 per cent and range from 13.0 per cent moisture at 80 per cent humidity for a spray powder from precondensed milk to 14.97 per cent moisture at 80 per cent humidity for roller powder from precondensed milk. One trial in which the sample was allowed to absorb 10 per cent moisture and then redried had a lower absorbing capacity when later exposed to a moist atmosphere.

EXPERIMENTAL PROCEDURE

The search of the literature reveals a large number of experimental methods available for investigating water sorption. Many are subject to severe criticism, and others are ill adapted to the investigation of dry milk solids. Those which have been used for dairy products seemed to fail to yield the type of information desired in this study. Because volume contraction as measured in a dilatometer is a method by which the progress of the reaction can be followed, and which lends itself readily to studying the effects of different fluids and agents, and which has been shown to be directly related to water sorption, it was selected as a method with which to study certain phases of this problem.

A description of the apparatus and method is detailed in an earlier paper (22). Briefly, the apparatus consists of a bulb flask attached to capillary tube and having side-arms for filling. The capillary tube has been calibrated with mercury. A quantity of dry milk solids is weighed into the flask, a few drops of toluene added as preservative, and the flask attached to the capillary by means of a ground glass joint. If it is desired to limit the amount of water, the proper amount is introduced through a side arm and the whole layered over with an immiscible oil extending into the capillary. If an unlimited amount of water to obtain maximum sorption is desired, as was the case in the experiments reported here, the flask is filled with water and the water is extended into the capillary. The apparatus is maintained isothermally in a water bath, under the conditions here reported at 20 degrees C. The height of the liquid is read at the beginning and at intervals thereafter until no further change occurs.

From the change in height of the liquid, the previously calibrated dimensions of the capillary, and the weight of dry material, the volume contraction per unit of solids can readily be calculated.

EXPERIMENTAL DATA

Samples of dry milk solids prepared by the spray process[1] and on the atmospheric roller dryer have been examined by this technique. These samples were allowed to remain in contact with water in the dilatometer until volume contraction had stopped. This yields maximum volume contraction. The values obtained in terms of cubic millimeters per gram of dry material for the differently processed samples are shown in the following table (Table 1).

TABLE 1

The maximum volume contraction of dry milk solids processed by different treatments

Sample no.	Moisture %	Acidity % reconstituted	pH reconstituted	Preheating temperature* C	F	Mfg. process	Volume contraction
1	3.8	0.165	6.80	60	140	Atm. Roll.	27 cu. mm./gm.
2	3.5	0.165	6.80	71.1	160	" "	31 " " "
3	3.2	0.165	6.80	76.7	170	" "	36 " " "
4	3.8	0.165	6.85	82.2	180	" "	30 " " "
5	3.1	0.165	6.80	93.3	200	" "	32 " " "
**6	3.7	0.160	6.80			" "	27 " " "
7	2.4	0.160	6.80	71.1	160	Spray	67 " " "
8	2.1	0.160	6.75	76.7	170	"	82 " " "
9	2.8	0.160	6.75	82.2	180	"	53 " " "
10	2.4	0.160	6.80	93.3	200	"	51 " " "

* Skim milk held at these temperatures for 30 minutes.
** Sample 6 is a commercial product prepared for the baking trade.

These results are shown graphically in the following chart (Figure 1).

The time relationships are of interest not only because they show the length of time necessary for maximum contraction to occur but also because they show the effect of processing treatments upon the rate of contraction. Figure 2 shows the data obtained with the samples prepared by the atmospheric roller process.

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[1] The samples of spray powder were prepared at the plant of the Milk Producers Association of Central California, Modesto, California, through the courtesy of the management.

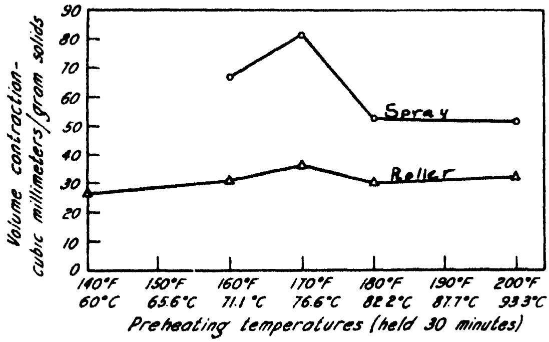

FIG. 1. The effect of preheating temperatures on volume contraction.

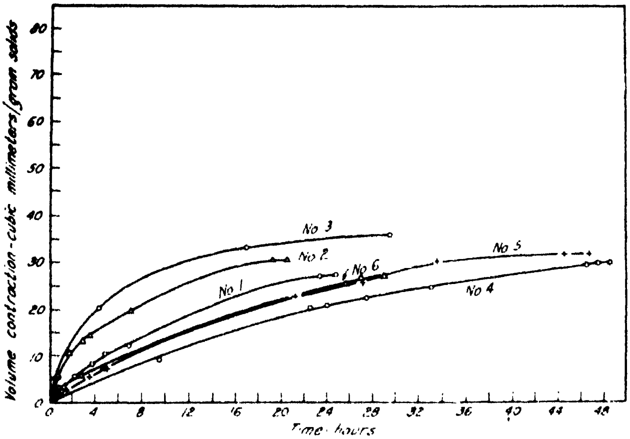

FIG. 2. The rate of volume contraction of atmospheric roller dry milk solids.

The information concerning the samples prepared by the spray process is shown in Figure 3.

It has been shown that the lactose present in dry milk solids prepared by the spray and roller processes is in the glass form unless the dried products are allowed to cake (23) in which case the lactose crystallizes in the alpha hydrate crystalline form (24), and in solution, lactose is present as an equilibrium mixture of the alpha and beta configurations (25). Since lactose undergoes these changes when exposed to water it seemed desirable to determine the effect of the change from the glass form to the crystalline form upon the volume contraction. Consequently, some dry milk solids from sample no. 8 were allowed to cake in a moist atmosphere and then redried to their original moisture content. There was no significant difference in the extent of volume contraction before and after caking, but there was a difference in the rate as shown in Figure 4.

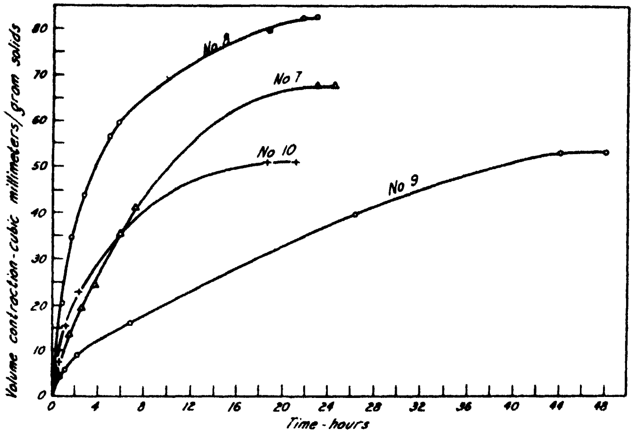

FIG. 3. The rate of volume contraction of spray process dry milk solids.

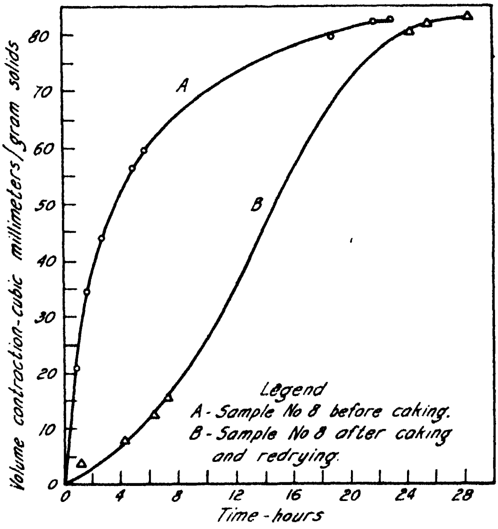

FIG. 4. The effect of caking and redrying on volume contraction.

DISCUSSION OF RESULTS

The data presented show only relative values for the degree of water sorption; work is in progress which will show the extent of volume contraction at different water levels. They do show, however, that different processing treatments produce noticeable effects in the properties of the dried materials.

Examination of Figure 1 shows that dry milk solids prepared by both processes show the same general reaction to the preheating treatment. There is a maximum contraction at 76.7 degrees C. (170 deg. F.) with each method, the effects being more marked with the spray process solids. It seems likely that the lower level of the roller process solids and the decreased values for the higher heat treatments are direct results of excess heat causing denaturation of the protein. There appears to be an optimum at about 76.7 degrees C. (170 deg. F.).

Figures 2 and 3 reveal some interesting comparisons. Again, the sequence of events occurs in the same order in the two types of dry milk solids and are more pronounced in the material by the spray process. The volume contraction is complete in about 20 to 22 hours for all of the spray samples except the one processed at 82.2 degrees C. (180 deg. F.) and in about 24 to 28 hours for the roller samples except the ones processed at 82.2 degrees C. (180 deg. F.) and 93.3 degrees C. (200 deg. F.). In both cases the 82.2 degrees C. (180 deg. F.) samples required about 48 hours for maximum volume contraction to occur. The roller sample preheated at 93.3 degrees C. (200 deg. F.) reacted somewhat faster than that processed at 82.2 degrees C. (180 deg. F.) but not as rapidly relatively as the 93.3 degrees C. (200 deg. F.) spray sample. Several trials were made in order to verify these results and no adequate explanation is available at this time. Heat denaturation would explain three of the four but the rapid rate of the 93.3 degrees C. (200 deg. F.) spray sample seemingly obviates this explanation.

The results shown in Figure 4 may be somewhat at variance from the data presented by Supplee (21) with which he showed that a sample which had been allowed to cake and was redried did not take up as much moisture from the air as it had previous to caking. However, it is known that colloidal materials will not take up the same amount of water from a moist atmosphere as they will from liquid water. The difference in the initial rate of contraction may result from the difference in the state of the lactose. If, in the original sample the lactose is in the form of a glass—a very highly supersaturated solution—this form would have a relatively high attraction for water and would wet easily; whereas, if in the redried sample the lactose is in the form of alpha hydrate, this form might not wet as easily, and since dry milk solids are approximately 50 per cent lactose the wetting of the whole and consequently the volume contraction might be delayed.

It is still debatable as to just what occurs in the volume contraction accompanying water sorption. The shrinkage may result from the compression of the solid resulting from a very strong attraction between the liquid and the solid, or it may result from a change in the association of the sorbed water with a change in specific volume. The latter explanation is generally regarded as the most probable one and, if true, it means a decrease in the specific volume of water of 30–40 per cent (6). On this basis the amount of water sorption can be calculated, and represents one per cent per gram of dry material for each three or four cubic millimeters contraction, depending upon which value is used for the specific volume of the water.

It remains to consider which constituents of milk are responsible for the volume contraction. It is well known that solution as such results usually in negligible volume changes. Preliminary experiments with alpha hydrate lactose by the method used for dry milk solids substantiated this for the lactose portion of the soluble milk constituents. It may be assumed that the mineral constituents act in the same manner. The fat content of the samples was approximately one per cent. In the light of Rishoi and Sharp's recent work (13) the volume change for this amount of fat at the temperature used is negligible. By elimination, the proteins, and possibly minor constituents such as phospholipids, are the only possibilities to contribute to the volume contraction. One may reasonably say then that the volume contraction of dry milk solids results mainly from water sorption by the milk proteins.

SUMMARY AND CONCLUSIONS

1. The volume contraction of dry milk solids prepared by different processing treatments immersed in an unlimited amount of water has been measured at 20 degrees C. in a dilatometer.
2. Dry milk solids preheated at 76.7 degrees C. (170 deg. F.) for 30 minutes before drying showed greater volume contraction in water than solids preheated at other temperatures studied, namely 60 degrees C. (140 deg. F.) (roller only), 71.1 degrees C. (160 deg. F.), 82.2 degrees C. (180 deg. F.), and 93.3 degrees C. (200 deg. F.).
3. Dry milk solids manufactured by the spray process showed greater volume contraction than when manufactured by the atmospheric roller process.
4. The time required for volume contraction to be complete ranged from 20–22 hours for all spray samples except the one processed at 82.2 degrees C. (180 deg. F.) and 24–28 hours for all roller samples except 82.2 degrees C. (180 deg. F.) and 93.3 degrees C. (200 deg. F.) to 48 hours for the samples processed by the higher heat treatments mentioned.
5. The significance of the data is discussed in the light of known facts concerning volume changes accompanying various phenomena and the conclusion is drawn that the volume contraction probably results mainly from the sorption of water by the milk proteins.

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THE SEPARATION OF FAT IN EVAPORATED MILK

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According to Stokes' Law governing the rate of rise of globules or the fall of particles in a medium, the rise of the fat is largely a function of the diameter of the fat globules and the viscosity of the dispersion medium.

Prior to the advent of the homogenizer evaporated milk was sterilized until a very heavy body was produced. This condition had a deterrent effect upon the velocity of rise of the fat but did not prevent it. In more recent years, decrease of the fat globule size through homogenization has permitted the production of a product of greater fluidity and with a decreased tendency to fat rising.

Low storage temperatures, which increase the viscous properties of the product, act as a deterrent to the rising of the fat. Shortening of the storage period and turning the cans at regular intervals are also practices often employed to prevent undue fat separation in evaporated milk.

While the effects of homogenization and viscosity on the rising of fat of evaporated milks have been known, apparently no actual measurements of the rate and the extent of separation have been published. Some observations upon this point will be presented in this article.

EXPERIMENTAL

The evaporated milk was made in 100-pound batches according to the usual commercial procedure. The milk was forewarmed at 95° C. for 10 minutes and concentrated in a small tinned-copper pan at 27 inches vacuum. The evaporated milk was homogenized either once or twice in a single stage machine, canned, and sterilized in a small pilot sterilizer.

Cans which held 160 grams of milk and which were $2\frac{1}{2}$ inches by $2\frac{1}{2}$ inches, outside dimensions, were used. The average height of the column of liquid in the can was $2\frac{3}{16}$ inches.

Viscosity measurements were made on the samples 24 hours after sterilization by means of a 25 cc. capillary viscosimeter. The time of flow into the pipette was taken for a series of pressures measured by means of a water manometer. A uniform pressure was obtained through the use of a 5-gallon reservoir.

The viscosity of the samples was calculated according to the proportion:

$$\frac{Vm}{Vs} = \frac{Dm \times tm}{Ds \times ts} \text{ wherein,}$$

Vm = viscosity of the milk

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TABLE 1

The effect of manufacturing processes and storage time upon the separation of fat in evaporated milk

Milk sample	Milk composition		Homogenization pressure	Sterilization treatment		Viscosity of sterilized milk	Butterfat in top third of can after storage at 28° C.				
	Total solids	Fat		Temperature	Time		21 days	46 days	87 days	141 days	252 days
	per cent	*per cent*	*pounds*	*°C.*	*min.*	*poises*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	26.10	8.3	1000	116	17	57.5	15.1	17.1	19.3	20.5	22.4
			2500			71.1	9.6	11.7	15.5	17.1	19.3
			2500–1000			71.2	8.8	11.8	14.3	16.0	18.8
2	27.70	7.9	1000	115	15	67.1	16.6	19.0	20.3	20.6	20.8
			2500			87.3	11.7	14.2	16.1	16.8	17.9
3	26.71	8.3	2500	115	15	48.4	12.1	14.6	16.6	18.2	19.5
			2500–1000			57.4	11.7	14.3	16.3	17.9	19.4
			4000			72.5	9.7	11.6	14.1	16.0	17.1
			4000–1000			77.0	9.6	11.2	13.1	15.9	17.2
			2500	115	18½	125.1	10.4	12.4	14.6	17.8	18.5

Vs = viscosity of a 60% sugar solution
Dm = density of milk
Ds = density of sugar solution
tm = time of flow of milk into pipette at the pressure of a 25-inch water column
ts = flow time for sugar solution at same pressure

With the exception of the last milk in the accompanying table (viscosity 125.1 poises), no sample showed a yield value. Correction was not made for yield value in calculating the viscosity of this sample.

The cans of sterilized milk were held undisturbed in storage in a room the temperature of which varied between 25° C. and 31° C. and which averaged 28° C.

Upon conclusion of the storage period, cans to be examined were removed very carefully, without tipping or shaking, to a cold room maintained at − 17.7° C. The next day the cans of frozen milk were opened, the solid milk cake removed and sliced into an upper, middle, and lower layer. Each layer was weighed and analyzed for fat.

Fat was determined by the Babcock method, modified by the addition of 1 cc. of normal butyl alcohol to each 9-gram charge of milk. It was found by comparison with the Röse-Gottlieb method that results obtained using this modified Babcock test showed a possible error of ± 0.1% fat.

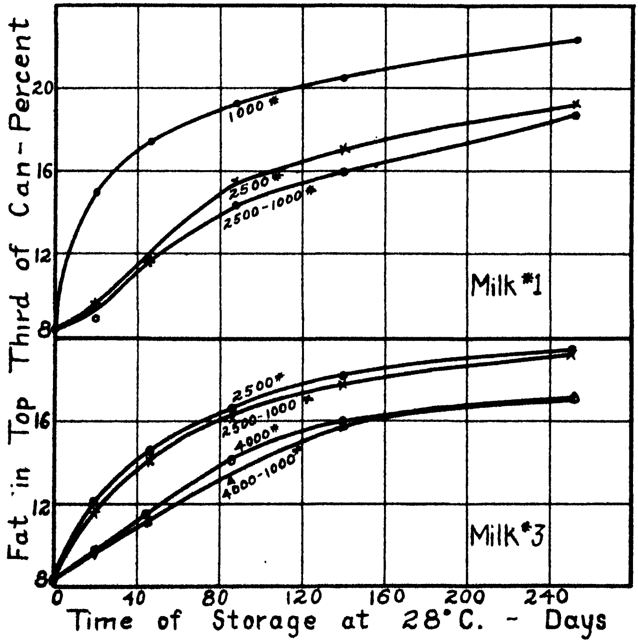

FIG. 1. The effect of storage time on the rise of fat in evaporated milks homogenized at different pressures.

Since the top layer was never exactly one-third, correction for this was made through the use of the weights and tests of the other layers. The corrected figures show the amount of fat which was present in the upper third part of the milk.

Fat separation data were secured on a number of different evaporated milks. Figures from 3 representative samples are given in the accompanying table. The differences between the fat content of the fresh evaporated milk and that of the top layer represents the fat which rose to the top from the lower two-thirds of the can. Data for milks 1 and 3 are plotted in Figure 1.

Although no means were found whereby fat separation could be prevented, this work indicates that effective methods of retarding separation are available for use in the manufacturing process. Two-stage homogenization with a high initial pressure and control of the sterilization process to produce a heavy-bodied milk were helpful in lessening the separation of fat in evaporated milk.

The influence of high viscosity or high homogenization pressures upon the distribution of fat is most noticeable during the first few weeks of storage. As the period of undisturbed storage continues, a noticeable decrease in the rate of fat separation occurs.

SUMMARY

A study of the distribution of fat in cans of evaporated milk during storage showed that high pressures of homogenization, two-stage homogenization, and high viscosity were effective in retarding separation.

SOME FACTORS INFLUENCING THE AMOUNT OF MOLD MYCELIA IN BUTTER[1]

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Mold mycelia in some food products are used as an index of the state of decomposition that existed in the original products before processing. In 1937, Wildman (1) introduced a test which determines, by the examination of butter, the approximate amount of mold contained in the cream from which the butter was manufactured. The technique of this test is similar to the method devised by Howard (2) for determining mold in tomato products. In the examination of butter the results are expressed as the per cent of fields examined which show mycelia fragments. A field is tabulated as positive when the total length of any two mycelia are equal to one-sixth the diameter of the microscopic field. It would appear, at first hand, that by enumerating the mold mycelia fragments in a sample of butter, much information could be obtained as to the age of the cream, temperature of storage, and care in production until manufactured into butter.

In order to obtain information on the significance of mold mycelia fragments in butter, a series of experiments were undertaken to determine: 1. Factors influencing the development of mold mycelia in cream as it may be handled on the producing farm. In this series of experiments raw cream was churned into butter using a laboratory churn. 2. The influence of some manufacturing methods on the mold mycelia content of butter. In this series of experiments pasteurized cream was used and the churnings made in a commercial churn unless otherwise specified.

I. FACTORS INFLUENCING THE DEVELOPMENT OF MOLD MYCELIA IN CREAM

Temperature and time of holding.

To secure information as to the effect of time and temperature of holding cream on the development of mold mycelia, fresh cream was secured from a clean separator, placed in cream cans and held for periods of from three to nine days at average temperatures of 60, 70, 75, and 80° F. To resemble farm conditions, at twelve-hour intervals equal additions of cooled cream were added to the accumulated supply. Twenty-eight trials were conducted to secure possible variations that may have existed in the micro flora of cream. The results obtained are presented in Table 1.

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[1] The data in this paper was taken in part from a thesis presented by Joseph Adams in partial fulfillment of the requirement for the Master of Science degree, and is published with the approval of the Director of the Agricultural Experiment Station.

* Industrial Fellowship of the Dairy and Ice Cream Machinery and Supplies Association.

TABLE 1

The influence of temperature and time of holding raw cream upon the mold mycelia content of the butter

Temperature of holding, °F.		Days held						
		3	4	5	6	7	8	9
		Percentage of positive fields						
60	Max.	4	15	22				27
	Av.	2	7	13				14
	Min.	0	0	6				10
70	Max.		6	16	44	74	88	96
	Av.		5	7	28	60	73	88
	Min.		4	5	17	46	58	80
75	Max.		24	25		84	100	100
	Av.		18	17		61	100	100
	Min.		10	8		53	100	100
80	Max.	10	100	100	100	100	100	100
	Av.	7	79	92	97	94	100	100
	Min.	3	56	72	86	81	100	100

The data show that temperature and time are important factors influencing the rate of growth of mold in cream. An average cream held at 70° F., or lower, for six days produced a butter that was positive for mold mycelia in less than 40 per cent of the fields examined, while cream held for four days at 80° F. produced a butter having 80 per cent of the fields positive. Considering maximum counts, butter with less than 40 per cent of the fields positive could be produced from cream coming from clean centrifugal separators when held for six days at 70° F., five days at 75° F., and three days at 80° F.

Surface area.

Molds are aerobic and their growth in cream is confined to the surface. Here their growth may be so abundant that a mat is formed and the mold becomes visible. Cream showing visible mold has been rejected by some cream graders. If, however, the mat is removed or stirred into the cream, the cream may then be acceptable. In order to determine the relation that exists between surface area of cream and the amount of mycelia fragments found in the butter, the following series of experiments were made. A volume of fresh sweet cream obtained from a clean separator was divided into three equal volumes and each was placed into a container of different diameter. Cream in container 1 had a surface area of 12.6 square inches, container 2, 17.7 square inches, and container 3, 58.7 square inches. An equal volume of cream was added to each container every 12 hours to imitate the method of handling cream on the farm. Twelve trials were conducted. The time and temperature of holding was constant in all trials.

As shown in Table 2, the amount of mold in cream, temperature and time being alike, is dependent upon the surface area of the cream in the container that is exposed to air. In general, as seen from the average figures,

TABLE 2

The influence of the surface area of the cream exposed to air upon the amount of mold mycelia in the resulting butter

Ratio of positive fields	Container 1	Container 2	Container 3
Maximum	1.0	2.0	10.0
Average	1.0	1.3	4.0
Minimum	1.0	1.0	1.2
Surface area of cream in sq. inches	12.6	17.7	56.7
Ratio of surface area	1.0	1.4	4.5

the amount of mold in a given sample of cream is almost directly proportional to the amount of cream surface exposed to air.

Agitation of cream during storage.

The general practice on farms is to take the separated cream, cool it, and then thoroughly mix the fresh cool cream into the stored cream. It is assumed that agitation would incorporate air into the cream and break mycelia clumps that may exist, thereby making conditions favorable for mold growth. In order to obtain data on the influence of agitation on the amount of mycelia in butter, the following conditions were followed.

Fresh sweet cream from a clean separator was added to the stored cream at twelve-hour intervals in equal quantities to cream cans having the same diameter. The added cream in one container was thoroughly mixed with a hand stirrer on each addition with the accumulated cream. In the other container, the fresh cream was carefully layered at each addition on to the accumulated cream and not mixed with the accumulated cream. These duplicate containers were then held for the same length of time and at the same temperature, the cream churned, and the butter examined for mold mycelia. The results secured are presented in Table 3.

TABLE 3

Increase of mold mycelia due to agitation during accumulation of cream

Increase in per cent of positive fields	No. of trials	Per cent of samples
0	6	17.2
1- 20	11	31.5
21- 40	8	22.8
41- 60	4	11.4
61- 80	4	11.4
81-100	2	5.7
Total no. of trials	35	

Of the 35 duplicate trials that were made, 82 per cent showed that the agitation of the cream during its accumulation on the farm caused an increase in the number of mold mycelia fragments in the butter. The six samples which showed no increase were samples that showed 100 per cent positive fields in both the non-agitated and agitated trials. Considering an arithmetical average, it was found that the agitated samples showed 67.5 per cent positive fields and the non-agitated 38.3 positive fields. No significant difference was apparent in the organoleptic qualities in the butter made from agitated and non-agitated cream. It does show that the stirring of cream favors the development of mold mycelia, but that it does not affect its grade.

Influence of the milk fat content of the cream upon the development of mycelia.

In unpublished results secured by Wildman, (3) he found, in the examination of cream as it was received by the creamery, that a trend was exhibited indicating that the amount of mycelia was greater in low testing cream than in high testing cream. This difference might have been due to the method of separation. However, in order to secure data, the following series of trials were conducted. Cream testing 45 per cent milk fat was secured from the separator. One-half of this cream was standardized with the skim milk obtained from this cream to a fat content of 15 per cent. The cream was then accumulated in identical containers and in the same manner held over varying time periods. In all, ten trials were made and the results are given in Table 4.

TABLE 4

Influence of milk fat content of cream upon mycelia development

Trial	Percentage of positive fields in the butter	
	15 per cent	45 per cent
1	6	4
2	98	75
3	100	56
4	99	100
5	10	4
6	66	15
7	10	8
8	100	86
9	24	14
10	76	72
Av.	59	37

The milk fat content of the cream was found to exert a slight influence upon mycelia development in cream, for in nine of the ten trials conducted, lower mycelia contents were obtained in the butter made from cream testing 45 per cent fat than from cream testing 15 per cent fat. The cream was obtained in both cases from the same source.

Limiting air supply to the cream surface.

The molds associated with cream and butter are aerobic and the bacteria causing cream deterioration are, for the most part, micro-aerophilic. Cream held under conditions which limit the air supply to the cream surface would show very little or no mold growth, but would show chemical changes due to bacterial growth. In order to obtain data upon the above condition, four trials were conducted in which identical samples of cream were held for six and twelve days under limited and unlimited air supply. The limited air supply to the cream was secured by covering the surface of the cream to a depth of one-fourth inch with heavy mineral oil. The cream was held at 75–80° F. and the results obtained are given in Table 5.

TABLE 5

Influence of limited air supply upon the mold mycelia development in cream as measured in the resulting butter

Trial	Per cent positive fields	
	Limited air	Unlimited air
	Six days	
1	0	63
2	0	81
3	0	50
4	0	72
	Twelve days	
1	0	100
2	0	100
3	0	100
4	0	100

The difference found in grading the butter obtained from the cream held for six and twelve days with limited and unlimited air supply showed that there was no significant difference in quality due to the inhibition of the molds by limiting the air supply. This again illustrates that the molds in cream are not the causative factor in deterioration of the cream and that a limited air supply to the cream surface inhibits the development of molds on the surface of the cream.

II. THE INFLUENCE OF SOME MANUFACTURING METHODS ON THE MOLD MYCELIA CONTENT OF THE BUTTER

Butter differs in some respects from other dairy products as in the process of manufacture, the fat is washed so that there usually remains less than one per cent of the original curd from the cream. In the churning process, factors exist which may influence the amount of mold retained in the butter and the distribution of mold in the butter mass. Thus, the problem arises as to the effect that some of the manufacturing processes will have upon the mold mycelia content of the butter.

Distribution of mycelia in butter.

To obtain data on the distribution of mycelia in butter, samples were taken from the churn at the gear end, drain end, and at the doors. Duplicate samples were taken and 100 fields were examined on each sample.

TABLE 6

Distribution of mycelia in a churning of butter

Churning	Gear end		Outlet end		Door 1		Door 2	
	Percentage of positive fields							
1	20	21	19	20	20	20	21	21
5	1	1	4	4	1	1	1	1
8	3	4	4	5	6	3	4	4
9	2	3	2	2	2	4	2	0

The data presented in Table 6, which are typical of the ten churnings examined, indicates that the mold mycelia in butter are uniformly distributed throughout the butter mass. Samples of low mycelia content are more significant than samples of high mycelia content, for the greater the amount of mycelia, the less the probable error in sampling and in distribution and it is for this reason that low mycelia content samples were selected.

The retention of the mycelia of cream in the butter made from that cream.

It has been demonstrated that but a small percentage of the bacteria found in the cream are retained in the butter, the majority being removed with the buttermilk and the wash water. No information was found in the literature indicating the retention of mold mycelia in the butter.

In order to secure data on the retention of mold mycelia in butter from cream, a method was devised to determine microscopically the amount of mycelia in cream. Samples of cream were obtained from commercial plants just prior to churning and of the finished butter. The number of samples examined were 121.

TABLE 7

Percentage retention of mold in butter as compared to the mold mycelia in cream

Percentage retention of mold in butter	No. of samples	Per cent of samples
Less than 10	29	24.0
11 - 20	26	21.5
21 - 30	25	20.7
31 - 40	20	16.5
41 - 50	12	10.0
51 - 60	6	5.0
61 - 70	3	2.5
Above 70	0	0.0

Table 7 indicates that of the molds found in cream, from less than ten per cent to as high as 70 per cent are retained in the butter, and that in 66.2 per cent of the samples the retention of mycelia was below 31 per cent. The amounts of mold mycelia in the butter varied from 0 to 100 per cent positive fields and no relation was found between the amount of mold in the cream and the per cent retained.

Under laboratory conditions, cream was inoculated with various strains of *Oospora lactis, Penicillium, Aspergillus,* and *Mucor;* the cream was not repasteurized after inoculation and was churned in a laboratory churn. The *Oospora lactis* strains were retained from nine to 42 per cent with an average of 23 per cent. The *Mucor, Aspergillus,* and *Penicillium* were found to be retained on the average of 10, 37, and 28 per cent, respectively.

These results indicate that the mold mycelia retention in butter of the mold mycelia in the cream is, in general, between 20 and 30 per cent.

Influence of size of butter granules.

Grimes (4), in studying factors affecting the retention of bacteria in butter, found that size of the butter granules were of importance, but in his work he made no mention of molds or mold mycelia. To obtain data upon this subject, samples were taken from the churn when the butter granules were about one-half the size of wheat grains and again when they were about twice as big as wheat grains. These samples were then analyzed for mold content using the plate and microscopic methods, and, as shown in Table 8, no significant difference was found in the mold plate count or in the microscopic mycelia count between underchurned or slightly overchurned butter.

TABLE 8

Influence of size of butter granules upon the mold content of butter

Trial	Granule size			
	One half wheat grains		Twice wheat grains	
	Mold per ml.	Per cent positive fields	Mold per ml.	Per cent positive fields
1	45,000	96	46,000	96
3	3,100	17	3,160	18
5	600,000	458*	500,000	560*
7	480,000	402*	500,000	524*
9	50,000	88	50,000	85
11	8,000	8	9,000	10

* Count of mycelia per 100 fields.

The degree of cream neutralization upon the mold mycelia content of butter.

Wildman (5), in studies on mold in cream, found that he was able to agglutinate the mold present in cream using a methylene blue borax solution, the pH of the solution being 9.2. In the process of butter manufacture from sour cream, the cream is neutralized to a pH between 6.0 and 7.0. In order to obtain data as to the effect of pH upon the amount of mold mycelia, six trials were conducted using sour cream which was neutralized to a pH of approximately 6.0–7.0 and to a pH of approximately 8.0. These creams were churned in a laboratory churn and then analyzed as to the per cent of fields positive for mold mycelia. The results which are given in Table 9 show that the pH limits to which cream is neutralized under commercial conditions do not influence the amounts of mold mycelia that are retained in the butter.

TABLE 9

Degree of cream neutralization upon the mold mycelia content of butter

Trial	Percentage of positive fields		
	Low pH	Medium pH	High pH
2	35	35	35
4	27	29	29
6	98	97	98

SUMMARY

1. Mold mycelia fragments in butter are an index of the length of time, temperature of storage, and the oxygen relationship of the cream from which the butter was made.
2. Mold mycelia in butter is not a direct index of the organoleptic quality of the cream from which the butter was made.
3. A higher percentage of mold mycelia is retained in the butter than bacteria. In general, the per cent retention is between 20 and 30 per cent.
4. Of the manufacturing operations studied none were found to significantly influence the mold mycelia content of the butter.

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HISTORY AND DEVELOPMENT OF THE STUDENTS' NATIONAL CONTEST IN THE JUDGING OF DAIRY PRODUCTS

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During the years which have intervened since the first intercollegiate students' dairy products judging contest held in 1916 in Springfield, Massachusetts, many records and data have been obtained regarding the dairy products judging contest, sponsored by the American Dairy Science Association.

Some of those data and records are herein assembled 1, that a permanent record of the more pertinent facts of the entire dairy products judging program may be had, and 2, that the changing personnel of coaches of dairy products judging teams and of dairy staff members may be informed concerning the development of dairy products judging.

To estimate the value of these students' national dairy products judging contests to the dairy industry is extremely difficult and, at best, figures would be only a matter of conjecture. Undoubtedly, the attainment of standards and ideals of perfection, both in the concrete and in the abstract, for butter, cheese, milk, and ice cream, have resulted from this contest, furnishing a clearing house of ideas and demonstrations to coaches and students alike.

COMMITTEES

The personnel of two committees of the American Dairy Science Association, the Committee for the Students' Dairy Products Judging Contest and the Committee for the Score Card for Dairy Products, should be noted particularly as being the guiding minds in the development of this contest. The personnel of these two committees throughout the period of the contests is presented in Tables 1 and 2. Thirteen different committees for the students' dairy products judging contest have been named.

The earlier committees of 1917, 1918, and 1919, chairmanned by Messrs. Lockwood, Thompson, and White, respectively, probably experienced the greatest difficulty in getting the contest under way. In his annual report before the American Dairy Science Association meeting, Mr. Lockwood[1] pointed out some of the problems confronting them:

> "The students' judging contest for dairy products was carried on under adverse conditions this year. . . . It cost more money to run our contest than to run the cattle judging contest. . . . It

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[1] J. DAIRY SC., 1: 425. 1917–18.

TABLE 1

Committees for the Students' Dairy Products Judging Contest, 1917–1938

1917[1] W. P. B. Lockwood, Ch. M. Mortensen C. E. Lee E. S. Guthrie H. C. Mills R. L. Lang	*1918*[2] S. C. Thompson, Ch. W. P. B. Lockwood M. Mortensen E. S. Guthrie C. Larson J. H. Frandsen	*1919*[3] William White, Ch. W. P. B. Lockwood M. Mortensen E. S. Guthrie C. Larson J. H. Frandsen S. C. Thompson
1920[4] William White, Ch. W. P. B. Lockwood A. W. Rudnick E. S. Guthrie J. H. Frandsen H. F. Judkins S. C. Thompson	*1922*[5] A. W. Rudnick, Ch. J. R. Keithley J. A. Gamble T. E. Wright R. B. Stoltz V. D. Chappell E. S. Guthrie S. C. Thompson	*1924*[6] H. A. Judkins, Ch. S. C. Thompson M. Mortensen G. D. Turnbow H. W. Gregory
1926[7] R. B. Stoltz, Ch. William White M. Mortensen G. D. Turnbow H. W. Gregory	*1927*[8] R. B. Stoltz, Ch. William White H. W. Gregory G. D. Turnbow P. A. Downs	*1932*[8] R. B. Stoltz, Ch. William White H. W. Gregory P. A. Downs H. A. Bendixen
1934[9] H. W. Gregory, Ch. William White P. A. Downs P. S. Lucas R. B. Stoltz, ex officio	*1936*[10] H. W. Gregory, Ch. R. B. Stoltz William White P. A. Downs R. W. Smith, Jr.	*1937*[11] H. W. Gregory, Ch. G. M. Trout P. A. Downs William White M. J. Mack
		1938[12] G. M. Trout, Ch. P. A. Downs William White M. J. Mack E. L. Fouts

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[1] J. Dairy Sc., 1, 439. 1917–18.
[2] J. Dairy Sc., 2, 61. 1919.
[3] J. Dairy Sc., 2, 433. 1919.
[4] J. Dairy Sc., 3, 237. 1920.
[5] J. Dairy Sc., 5, 199. 1922.
[6] J. Dairy Sc., 7, 115. 309. 1924.
[7] J. Dairy Sc., 9, 503. 1926.
[8] Letters, R. B. Stoltz.
[9] J. Dairy Sc., 17, 340. 1934.
[10] J. Dairy Sc., 19, 223. 1936.
[11] J. Dairy Sc., 20, 65. 1937.
[12] J. Dairy Sc., 21, 57. 1938.

would seem that the judging of dairy products deserves pretty nearly as good a place as the judging of cattle, and we can get it; but the attitude is not towards it; and we will have to create this attitude. . . . When we find a high grade of butter, score it, discuss it, and carry back to our institutions the ideas and standards that we will have to meet in our production. . . . I believe manufacturers will put up a manufacturer's scholarship. Possibly a

TABLE 2

Committees for the Score Card for Dairy Products, 1918–1926

1918[1]	*1919*[2]	*1920*[3]
J. H. Frandsen, Ch.	J. H. Frandsen, Ch.	J. H. Frandsen, Ch.
H. B. Ellenberger	H. B. Ellenberger	H. B. Ellenberger
M. Mortensen	M. Mortensen	S. C. Thompson
S. C. Thompson	S. C. Thompson	B. W. Hammer
Ernest Kelly	Ernest Kelly	L. A. Rogers
W. P. B. Lockwood	W. P. B. Lockwood	Ernest Kelly
W. W. Fisk	W. W. Fisk	W. P. B. Lockwood
J. L. Sammis	J. L. Sammis	W. W. Fisk
		J. L. Sammis
		O. F. Hunziker

1922[4]	*1924*[5]	*1926*[6]
J. H. Frandsen, Ch.	J. H. Frandsen, Ch.	J. H. Frandsen, Ch.
H. B. Ellenberger	O. F. Hunziker	O. F. Hunziker
S. C. Thompson	L. A. Rogers	L. A. Rogers
B. W. Hammer	J. A. Gamble	J. A. Gamble
L. A. Rogers	W. P. B. Lockwood	W. P. B. Lockwood
J. A. Gamble		
Ernest Kelly		
W. P. B. Lockwood		
W. W. Fisk		
J. L. Sammis		
O. F. Hunziker		
A. C. Baer		

[1] J. Dairy Sc., 2, 61. 1919.
[2] J. Dairy Sc., 2, 433. 1919.
[3] J. Dairy Sc., 3, 237. 1920.
[4] J. Dairy Sc., 5, 199. 1922.
[5] J. Dairy Sc., 7, 309. 1924.
[6] J. Dairy Sc., 9, 503. 1926.

> group of manufacturers, or interested men, may be persuaded to contribute a small amount each for its support.''

Interest in the contest continually increased through the chairmanships of Messrs. White, Rudnick, Judkins, Stoltz, and Gregory. During the tenure of Professor Stoltz' committee, which served the period of longest duration, from 1926 to 1934, the visions of Professor Lockwood came to fruition in the form of more favorable conditions for scoring, incentives, financial support, and industrial fellowships. To Professors Stoltz' and Gregory's committee fell largely the responsibility of administering those research fellowships.

A survey of the committees presented in Table 1 shows the name of William White as serving the students' judging contest project for the longest period of time. Although his name does not appear on the committees named in 1922 and 1924, it must be noted that during this time he was superintendent of the national contest (Table 7).

The second committee whose action has had a profound influence upon the present scoring of dairy products and which has worked in collaboration with the Committee for the Students' Dairy Products Judging Contest is

TABLE 3

Colleges participating in the Students' National Dairy Products Judging Contest by years, 1916–1938

No.	College	'16	'17	'19	'20	'21	'22	'23	'24	'25	'26	'27	'28	'29	'30	'31	'32	'33	'34	'35	'36	'37	'38	No. of contests in which teams participated
		Year in which team was entered in contest																						
1	University of Arkansas					*												*	*	*				4
2	University of California								*		*				*									3
3	Connecticut State College	*						*			*	*			*	*	*	*	*	*	*	*	*	13
4	Cornell University	*		*				*									*	*	*	*	*	*	*	10
5	University of Georgia												*	*					*					3
6	University of Illinois														*	*	*	*	*	*	*	*	*	9
7	Iowa State College			*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	19
8	Kansas State College										*	*	*	*	*	*	*	*	*	*	*	*	*	13
9	University of Louisiana																*					*		2
10	Maryland State College			*	*			*									*				*		*	6
11	Massachusetts State College	*					*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	18
12	Michigan State College									*	*	*	*	*	*	*	*	*	*	*	*	*	*	14
13	University of Minnesota					*	*									*	*	*	*	*	*	*	*	10
14	Mississippi State College											*	*	*	*	*	*	*	*	*	*	*	*	12
15	University of Nebraska	*	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	20
16	University of New Hampshire	*						*				*												3
17	New Mexico State College																						*	1
18	University of North Carolina										*	*	*											3
19	Ohio State University	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	21
20	Oklahoma A. & M. College												*	*				*	*	*			*	6
21	Ontario Agricultural College														*	*					*		*	4
22	Oregon Agricultural College						*		*	*	*	*	*	*	*									8
23	Pennsylvania State College	*				*		*	*	*	*	*			*	*			*		*		*	12
24	Purdue University			*	*		*		*	*			*	*	*		*	*	*	*	*	*	*	15
25	Rutgers University															*					*			2
26	South Dakota State College	*	*	*	*	*	*			*	*	*	*	*			*	*	*	*		*	*	17
27	University of Tennessee											*	*	*	*				*	*	*	*	*	9
28	Texas Technological College																					*	*	2
29	University of Vermont	*														*	*						*	4
30	Virginia Polytechnic Institute													*		*								2
31	University of West Virginia					*	*		*	*	*	*	*	*	*	*	*	*					*	13
32	University of Wisconsin								*						*		*	*	*	*	*	*	*	9
	Total	9	3	7	5	8	9	7	10	10	13	15	14	15	17	16	18	17	19	17	18	17	23	287

Note: A contest was not held in 1918 because of the World War.

the Committee for the Score Card for Dairy Products. Of the six committees named from 1918 to 1926, Professor J. H. Frandsen has served as chairman throughout. A glance at the names of the personnel of these committees reveals the names of many leaders in the field of dairy manufacturing today, all of whom have contributed toward the development of the score cards now used in the scoring of butter, cheese, milk, and ice cream. The men who served on these committees at various times were: Messrs. Frandsen, Ellenberger, Mortensen, Thompson, Kelly, Lockwood, Fisk, Sammis, Hammer, Rogers, Hunziker, Gamble, and Baer.

NUMBER OF CONTESTS

The Students' National Contest in the Judging of Dairy Products has been held yearly since 1916, with the exception of the year 1918 when a contest was not sponsored because of the World War. From 1916 to 1938 inclusive, 22 separate contests have been held to which 32 colleges have sent 287 teams of three men each, a total of 861 contestants. In addition, approximately 225 alternates have accompanied these teams, and likewise have had the opportunity of witnessing the exposition with which the contest was affiliated, as well as studying the products assembled for judging.

COLLEGES REPRESENTED

The names of the colleges competing in the various contests are presented alphabetically in Table 3. An examination of the data in this table shows that 13 colleges have entered teams in the judging event in 10 or more

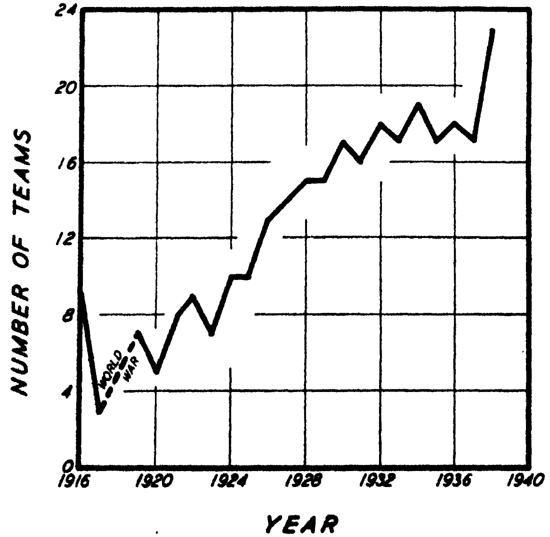

FIG. 1. The growth of the National Dairy Products Judging Contest.

of the 22 national contests. Teams representing Ohio State University have participated in 21 of the 22 contests, missing only one year, 1927. Nebraska, Iowa, Massachusetts, and South Dakota have been represented in all of the events but two, three, four, and five respectively. The growth of the Students' National Dairy Products Judging Contest with the years can best be seen in Figure 1. Here it will be noted that increasing interest, judging from the number of teams entered began to be manifest about 1926, continued to increase gradually until 1934, when, up to that time, the highest number, 19 teams, were entered in the contest. Four years later, 1938, 23 teams participated in the event.

Since 1930, when the Students' National Contest in the Judging of Dairy Products was sponsored jointly for the first time by the American Dairy Science Association and the Dairy Industries Supply Association, Inc., the team entries have averaged 18 per year.

During the 22 years since the inception of the judging project the Students' National Dairy Products Judging Contest has been held in 13 different cities located in the eastern half of the United States and has been affiliated with two separate organizations, namely, the National Dairy Association and the Dairy Industries Supply Association, Inc. The location, year, and number of colleges represented yearly are shown in Table 4.

TABLE 4

Location of the Students' National Dairy Products Judging Contest, 1916–1938

Number of contest	Year	Organization with whom affiliated	Location	No. of teams
1	1916	National Dairy Association	Springfield, Mass.	9
2	1917	" " "	Columbus, Ohio	3
3	1919	" " "	Chicago, Ill.	7
4	1920	" " "	Chicago, Ill.	5
5	1921	" " "	St. Paul, Minn.	8
6	1922	" " "	St. Paul, Minn.	9
7	1923	" " "	Syracuse, N. Y.	7
8	1924	" " "	Milwaukee, Wis.	10
9	1925	" " "	Indianapolis, Ind.	10
10	1926	" " "	Detroit, Mich.	13
11	1927	" " "	Memphis, Tenn.	14
12	1928	" " "	Memphis, Tenn.	15
13	1929	" " "	St. Louis, Mo.	15
14	1930	Dairy Industries Supply Association	Cleveland, Ohio	17
15	1931	" " " "	Atlantic City, N. J.	16
16	1932	" " " "	Detroit, Mich.	18
17	1933	" " " "	Chicago, Ill.	17
18	1934	" " " "	Cleveland, Ohio	19
19	1935	" " " "	St. Louis, Mo.	17
20	1936	" " " "	Atlantic City, N. J.	18
21	1937	" " " "	New Orleans, La.	17
22	1938	" " " "	Cleveland, Ohio	23

OFFICIAL JUDGES

The placing of the official scores and criticisms on the individual samples of butter, cheese, milk, and ice cream used in judging has been a real responsibility. Much of the success of a dairy products judging program is dependent upon the establishment of standards, having a continuity from year to year. The standards for flavors, bodies, textures, and so on established one year in a contest furnished mental standards to the coach for training students the following year. Therefore, the placing of scores upon dairy products used in the contest has had a wide and profound influence in raising the level of quality of dairy products nationally.

The task of selecting qualified judges has fallen largely to Mr. William White, Bureau of Dairying, United States Department of Agriculture, who has been superintendent of the National Dairy Products Judging Contest since 1925. The judges selected to place the official scores on the various products are listed in Table 5. Only the names of the judges since and including 1926, the year in which ice cream was first a part of the judging program, are included. Perhaps the most striking feature of this table is the recurrence of the name of the official judge of a product year after year. The use of the same judge for a number of years, especially in the scoring of a product in which the national standards are not so well established, would seem to be the part of wisdom. By this procedure, the scoring of cheese, milk, and ice cream has become more standardized than formerly. The comparative uniformity of butter scoring throughout the country has been recognized for some time.

TABLE 5

The judges who placed the official scores and criticisms on the samples of butter, cheese, milk, and ice cream, in the National Dairy Products Judging Contest since 1926

Year	Butter	Cheese	Milk	Ice cream
1926	L. E. Gaylord	C. W. Freyhoffer	R. J. Posson	H. F. Judkins
	H. W. Gregory	E. L. Aderhold	C. E. Clement	C. S. Trimble
1927	L. S. Edwards	G. N. Tobey	R. J. Posson	H. F. Judkins
	G. A. Gilbert	G. A. Gilbert		
1928	G. A. Gilbert	G. A. Gilbert	C. J. Babcock	W. H. E. Reid
	L. S. Edwards	G. N. Tobey	R. W. Bell	
		L. H. Marlatt		
1929	H. D. Reynolds	H. L. Wilson	C. J. Babcock	W. H. E. Reid
	L. S. Edwards	William White	C. S. Leete	P. H. Tracy
1930	O. A. Storvick	H. L. Wilson	Ernest Kelly	A. C. Dahlberg
	H. D. Reynolds	J. W. Moore	C. J. Babcock	A. D. Burke
1931	L. D. Reekie	H. L. Wilson	C. J. Babcock	A. C. Dahlberg
	L. S. Edwards	W. E. Ayers	F. M. Grant	A. D. Burke
1932	C. E. Eckles	H. L. Wilson	C. J. Babcock	A. C. Dahlberg
1933	C. L. Pier	H. L. Wilson	C. J. Babcock	A. C. Dahlberg
1934	L. S. Edwards	H. L. Wilson	C. J. Babcock	A. C. Dahlberg
1935	L. S. Edwards	H. L. Wilson	C. J. Babcock	A. C. Dahlberg
1936	L. S. Edwards	H. L. Wilson	C. J. Babcock	A. C. Dahlberg
1937	L. S. Edwards	H. L. Wilson	C. J. Babcock	A. C. Dahlberg
1938	L. S. Edwards	H. L. Wilson	C. J. Babcock	A. C. Dahlberg

Since 1932 the official scoring of each product has been checked by two coach judges appointed by the Dairy Products Judging Committee. The names of the coach judges are presented in Table 6. These coach judges working independently rescored the samples. If a comparison of scores revealed a total lack of agreement between the official judge and the coach judge as to the evaluation of scores or criticisms, then the sample in question was not used in the contest. Each year eight coach judges, two for each product, have thus assisted in the official judging since the introduction of this system.

TABLE 5a

Identification of dairy products judges

Ice Cream

A. D. Burke, Alabama Polytechnic Institute, Auburn, Ala.
A. C. Dahlberg, N. Y. State Agr. Expt. Sta., Geneva, N. Y.
H. F. Judkins, Eastern States Dairies, Springfield, Mass.
W. H. E. Reid, University of Missouri, Columbia, Mo.
P. H. Tracy, University of Illinois, Urbana, Ill.
C. S. Trimble, Bureau of Dairy Industry, Washington, D. C.

Butter

L. S. Edwards, Bureau of Dairy Industry, Nashville, Tenn.
L. E. Gaylord, Bureau of Agricultural Economics, Washington, D. C.
G. A. Gilbert, U. S. Food Products Inspector, Chicago, Ill.
H. W. Gregory, Purdue University, Lafayette, Ind.
H. D. Reynolds, Mason City, Iowa.
O. A. Storvick, Albert Lea, Minn.
L. D. Reekie, Bureau of Agricultural Economics, New York City.
C. E. Eckles, Bureau of Agricultural Economics, Washington, D. C.
C. L. Pier, Bureau of Agricultural Economics, Chicago, Ill.

Cheese

E. L. Aderhold, Neenah, Wis.
W. E. Ayers, Cornell University, Ithaca, N. Y.
C. W. Freyhoffer, Bureau of Agricultural Economics, Washington, D. C.
G. A. Gilbert, U. S. Food Products Inspector, Chicago, Ill.
L. H. Marlatt, Cheese Specialist, Athens, Ga.
J. W. Moore, Dept. of Agr. and Markets, Madison, Wis.
G. N. Tobey, Cheese Specialist, Knoxville, Tenn.
Wm. White, Bureau of Dairy Industry, Washington, D. C.
H. L. Wilson, Bureau of Dairy Industry, Washington, D. C.

Milk

C. J. Babcock, Bureau of Dairy Industry, Washington, D. C.
R. J. Bell, U. S. Dept. of Agri., Washington, D. C.
C. E. Clement, Bureau of Dairy Industry, Washington, D. C.
Ernest Kelly, Bureau of Dairy Industry, Washington, D. C.
C. S. Leete, Bureau of Dairy Industry, Washington, D. C.
R. J. Posson, Bureau of Dairy Industry, Washington, D. C.
F. M. Grant, Bureau of Dairy Industry, Washington, D. C.

SUPERINTENDENTS OF CONTESTS

From the inception of student dairy products judging to the present time only two men have superintended the contest, as shown in Table 7. These

TABLE 6

The coach judges who have assisted in the official scoring of the samples of butter, cheese, milk, and ice cream used in the National Dairy Products Judging Contest

Year	Butter	Cheese	Milk	Ice Cream
1932	E. S. Guthrie R. E. Roberts	W. H. Martin E. F. Goss	G. M. Trout L. M. Thurston	P. H. Tracy R. W. Smith
1933	L. C. Thomsen F. H. Herzer	P. A. Downs G. M. Trout	L. H. Burgwald H. G. Lindquist	C. A. Iverson E. L. Fouts
1934	M. Mortensen R. E. Roberts	F. W. Bennett C. A. Jacobson	E. O. Anderson S. T. Coulter	W. H. Martin J. H. Erb
1935	S. L. Tuckey E. S. Guthrie	R. E. Roberts S. T. Coulter	W. H. Martin E. L. Fouts	F. H. Herzer P. S. Lucas
1936	L. C. Thomsen C. M. Mecham	H. G. Lindquist W. H. Sproule	P. H. Tracy F. J. Doan	L. R. Dowd P. A. Downs
1937	N. E. Fabricius R. E. Roberts	S. T. Coulter S. L. Tuckey	T. B. Harrison H. G. Lindquist	F. H. Herzer G. M. Trout
1938	E. O. Herreid S. T. Coulter	L. C. Thomsen K. R. Renner	I. A. Gould F. J. Doan	W. H. Martin N. E. Fabricius

honors have been shared by Mr. S. C. Thompson and Mr. William White of the Bureau of Dairy Industry, United States Department of Agriculture. Mr. S. C. Thompson was the superintendent for the first two years; Mr. White for the next two; and then Mr. Thompson was superintendent again until his death in 1925. Since then, for 13 years, Mr. William White, Senior Dairy Manufacturing Specialist, Bureau of Dairy Industry, United States Department of Agriculture, has been the superintendent of the contest.

TABLE 7

Superintendents of National Dairy Products Judging Contest

Year	Name	Year	Name
1916	S. C. Thompson	1928	William White
1917	" " "	1929	" "
1919	William White	1930	" "
1920	" "	1931	" "
1921	S. C. Thompson	1932	" "
1922	" " "	1933	" "
1923	" " "	1934	" "
1924	" " "	1935	" "
1925	William White	1936	" "
1926	" "	1937	" "
1927	" "	1938	" "

RANKING OF COLLEGES IN JUDGING DAIRY PRODUCTS

A summary of the ranking of the colleges in the judging of dairy products is presented in Table 8. Since 1926, four dairy products, butter, cheese, milk,

TABLE 8

Summary of ranking colleges in the judging of dairy products

Year	Colleges winning first place in dairy products judging, by years					No. teams per year
	Butter	Cheese	Milk	Ice Cream	All products	
1916	Pennsylvania*					9
1917	S. Dakota	Nebraska	S. Dakota	**	***	3
1918	****					..
1919	Iowa	Ohio	S. Dakota		S. Dakota	7
1920	S. Dakota	Ohio	Ohio		Ohio	5
1921	Ohio	Ohio	Ohio		Ohio	8
1922	S. Dakota	Ohio	Massachusetts		Ohio	9
1923	Pennsylvania	Ohio	Ohio		Pennsylvania	7
1924	Iowa	Purdue	Massachusetts		Iowa	10
1925	S. Dakota	Iowa	W. Virginia		Iowa	10
1926	Oregon	S. Dakota	Iowa	Iowa	Iowa	13
1927	Iowa	Tennessee	Kansas	Massachusetts	Iowa	14
1928	Iowa	W. Virginia	S. Dakota	Ohio	Iowa	15
1929	Oregon	Ohio	Ohio	Iowa	Ohio	15
1930	Mississippi	Kansas	W. Virginia	Illinois	Kansas	17
1931	Mississippi	Michigan	Iowa	Vermont	Iowa	16
1932	Purdue	Michigan	Purdue	Mississippi	Mississippi	18
1933	S. Dakota	Wisconsin	Illinois	Massachusetts	Ohio	17
1934	Minnesota	Iowa	Mississippi	Ohio	Ohio	19
1935	Tennessee	Mississippi	Tennessee	Nebraska	Mississippi	17
1936	Iowa	Tennessee	Ohio	Cornell	Ohio	18
1937	Nebraska	Minnesota	S. Dakota	Ohio	Ohio	17
1938	Iowa	Cornell	Ohio	Cornell	Cornell	23

* Butter only judged in 1916.

** Ice Cream not included until 1926.

*** No rating for all products.

**** No contest because of World War.

and ice cream have been judged, ice cream being included that year for the first time. Butter only was judged in the first contest in 1916, while cheese and milk were first included in the second scoring contest, in 1917.

Of the 32 colleges which have been represented in the judging event, only seven, namely, Ohio, Iowa, Mississippi, South Dakota, Pennsylvania, Kansas, and Cornell, have won sweepstakes honors one or more times. Ohio State University has won first eight times; Iowa State College, six times, five years consecutively from 1924 to 1928 inclusive; Mississippi State College, twice; and the State Colleges of South Dakota, Pennsylvania, Kansas, and Cornell University each once; Ohio State University teams have won the sweepstakes position 38.1 per cent of the contests entered, 21 times, while Iowa State teams have won 31.6 per cent of the contests entered, 19 times.

In the judging of butter, Iowa has won first place six times to South Dakota's five. Mississippi, Oregon, and Pennsylvania, each have won the butter judging contest twice, while Ohio, Purdue, Minnesota, Tennessee, and Nebraska each have won the contest once.

Teams representing Ohio State University have won the cheese judging contest six times, winning five years consecutively from 1919 to 1923 inclusive. Iowa, Michigan, and Tennessee each have won twice, while Nebraska, Purdue, South Dakota, West Virginia, Kansas, Wisconsin, Mississippi, Minnesota, and Cornell have enjoyed the honor once.

Milk judging sweepstakes have gone to Ohio six times; to South Dakota four; to Massachusetts, West Virginia, and Iowa, twice; and to Kansas, Purdue, Illinois, Mississippi, and Tennessee once.

In judging ice cream, the Ohio State University teams have won first place three times of the 13 years in which ice cream judging was included in the contest. Iowa, Massachusetts, and Cornell each have won twice, while Illinois, Vermont, Mississippi and Nebraska each have won once.

AFFILIATION WITH THE DAIRY INDUSTRIES SUPPLY ASSOCIATION, INC.

The year 1930 marked the beginning of a new era in the student judging of dairy products for in that year for the first time the National Students' Dairy Products Judging Contest was sponsored jointly by the American Dairy Science Association and the Dairy Industries Supply Association, Inc., and was held in connection with the Dairy Industries Exposition. Early in that year representatives of the students' dairy products judging committee, of which Professor Stoltz was chairman, met with representatives of the committee of the Dairy Industries Supply Association, Mr. Roberts Everett, Executive Vice-President, and Mr. C. Earl Breece, Secretary, to evolve a plan whereby each organization might be more ably served by a joint dairy products judging program.

As a result of the program projected, nine national students' dairy products judging events have now been held jointly by these two associations in six different cities throughout the eastern half of the United States. The consistently high team participation regardless of the location of the contest, as shown in Table 4 and Figure 1, attest to the interest awakened in students' dairy products judging through adequate incentives, which were provided as a result of the long time program outlined by the committees of these two associations.

INCENTIVES

From 1930 to 1938 inclusive, 162 teams of three men each, a total of 486 team members, plus approximately 125 alternates participated in the judging event activities. Regardless of the location of the contest or of the general business condition of the country, no less than a low of 16 teams and a high of 23 teams, with an average of 18 teams, competed yearly.

A study of these contests indicates quite clearly that at least four incentives have had an influence upon the maintenance of this high team participation. These are 1, the dairy manufacturing student had an opportunity of visiting the Dairy Industries Exposition in addition to the contest; 2, financial aid made possible greater facilities, comfort, and more pleasing environment for judging dairy products; and 3, the honors evening banquet devoted entirely to dairy manufacturing students furnished a setting whereby the student could get a vision of, or the sense of belonging to, the dairy industry; and 4, awarding of the coveted industrial fellowships made possible further educational opportunities.

The Dairy Industries Supply Association has made possible largely these incentives through its hearty cooperation and financial support. During the past nine years this association has awarded 51 fellowship grants totaling $34,200 00, as shown in Table 9, to 51 students showing proficiency in scholarship as well as in the judging of dairy products. In addition, the Association has borne in large part the expense connected with the actual products judging as well as the honors evening banquet.

TABLE 9

Fellowship grants of the Dairy and Ice Cream Machinery and Supplies Association, Inc., 1930–1938

Year	Number of grants	Value of each	Total
1930	6	$750.00	$ 4,500.00
1931	6	750.00	4,500.00
1932	6	750.00	4,500.00
1933	6	750.00	4.500.00
1934	6	600.00	3,600.00
1935	6	600.00	3,600.00
1936	6	600.00	3,600.00
1937	6	600.00	3,600.00
1938	3	600.00	1,800.00
Total	51		$34,200.00

COLLEGES COMPETING 1930–1938, INCLUSIVE

A total of 30 different colleges have participated in the national dairy products judging contest since 1930. Nine colleges, Connecticut, Iowa, Illinois, Kansas, Massachusetts, Michigan, Mississippi, Nebraska, and Ohio have entered teams each year, while six other colleges have entered teams six or more years during the nine years of the jointly sponsored contest.

FELLOWSHIP WINNERS

A roster of the colleges winning one or more of the Dairy Industries Supply Association Fellowships contains the names of 17 colleges sharing those honors from 1930 to 1938, inclusive. These colleges are scattered over 16 states and the Province of Ontario, Canada.

Colleges from Ohio, Mississippi, and Minnesota have been awarded seven, six, and five fellowships respectively, while those from Iowa, Kansas, Michigan, and Nebraska each have been awarded four fellowships. Cornell has won three, while Connecticut, Illinois, Massachusetts, South Dakota, and

Tennessee each have won two fellowships. Arkansas, Ontario, Purdue, and Vermont each have won one fellowship.

From 1930 to 1933 inclusive the fellowships were awarded to the ranking individuals in the judging of dairy products. Since 1934, the fellowships have been awarded to the schools leading in judging, and have been granted to the team member or alternate selected, largely on the basis of scholarships, by a committee from that school.

The research under these grants has been carried out in 14 state colleges or universities, namely, California, Connecticut, Cornell, Iowa, Illinois, Kansas, Massachusetts, Michigan, Minnesota, Nebraska, Ohio, Pennsylvania, Purdue, and Wisconsin. Considering the 17 schools winning the fellowships with the 14 schools to which Fellows have been assigned for graduate study, a total of 20 different institutions have felt the influence of these grants at one time or another. Several institutions have won both a fellowship and have been assigned a fellow from another institution the same year.

ULTIMATE SUCCESS OF DAIRY PRODUCTS JUDGING CONTESTS

Based upon the number of contestants in dairy products judging during the past decade, the National Dairy Products Judging Contest would seem to have succeeded beyond the dream of those responsible for its initiation. Yet numbers alone probably are a poor criteria by which to evaluate the success of the project. Rather the consciousness in dairy products judging awakened by these contests and a recognition of standards of quality throughout the country would be a more accurate measure of its worth. Today, in several states, the judging of dairy products is carried into the Smith-Hughes schools, where high school boys and girls are becoming conscious of quality in dairy products. If the National Dairy Products Judging Contest has furnished the incentive for training leaders to carry on such a phase of the quality program the time and effort have been well expended.

American Dairy Science Association Announcements

THIRTY-FOURTH ANNUAL MEETING

Plans are being completed for the housing and entertainment of the members attending the 34th Annual Meeting of the American Dairy Science Association to be held at Pullman, Washington, and Moscow, Idaho, June 27–30, 1939. Questionnaires are being sent out giving an opportunity to all parties to make the type of reservations desired by them. Accommodations of every kind will be available, including modern college dormitories, hotels, tourist cabins. To facilitate arrangements, it is requested that reservations for accommodations be returned before June 1.

THE COMPLETE PROGRAM WILL BE FOUND IN THE JUNE NUMBER OF THE JOURNAL

A large attendance is expected. Bring the family. Free guest tickets will be provided for members to play golf in Pullman and in Moscow. Tennis courts and swimming pools will be open to our guests. Fishing should be good in the not too distant mountain streams for the friends of Isaac Walton who wish to relax after the meetings. Plan now for a glorious trip to the beautiful Pacific Northwest. Paved highways all the way out. Grand scenery, cool nights. See the great Coulee Dam, large cooperative plants, fine herds, Mt. Rainier and other National Parks. Westward ho!

The distance from Pullman to Moscow is 9 miles on a good highway. It takes less than 15 minutes to drive between the two institutions.

CANADIAN SOCIETY OF TECHNICAL AGRICULTURISTS

Mr. H. L. Trueman, Secretary of the Canadian Society of Technical Agriculturists, informs us that the C. S. T. A. Meeting and the Annual Convention is to be held in Vancouver, British Columbia, from June 19–22. This makes it possible for those interested to attend both conventions.

LETTER FROM THE SECRETARY

Members:

Are you planning to attend the annual meeting in June? Many members and subscribers are making plans now to kill two birds with one stone.

First—Attend the meeting and rub shoulders with other dairymen who are attempting to do the same thing for which we are all striving. Recharge the storage battery to retain enthusiasm. Get new ideas. Go home and do a

better job. Our progress in the future will depend more and more upon the new developments and more efficient short cuts than ever before.

Second—Give yourself and your wife or family a peep at the great U. S. You have never been any place if you haven't seen the Great Northwest.

It's not my intention to oversell you, but before you live another year you should have the privilege of associating with the people of the great United States in their native, outspoken, hospitable and courteous way. You will want to see the beautiful Western Country, the mountains, and the National Parks. Most people find it necessary to sacrifice something at home to see these things. It's worth it to most of those who go. The cost is low.

You need a change, your wife needs a change of scenery and your children are the correct age to be educated.

Some will choose to drive; others will go on the train. The round trip rail fare from Chicago, including sleepers, is from $80.40 to $110.55. The Northern Pacific Railway has been selected as the official route so that those going by train may leave Chicago traveling together from Thursday, June 22nd, until Monday, June 26th.

I have been told that there is good fishing in the Northwest. Usually, however, one is so busy seeing things that one doesn't have time to fish.

We trust that many families will be able to take in this wonderful trip.

Sincerely yours,
R. B. Stoltz, Sec'y-Treas.

NEW MEMBERS FOR 1939—BY STATES

APPLICATIONS RECEIVED BETWEEN MARCH 6–APRIL 10, 1939

ARKANSAS

Gregg, V. L., ***Chairman, State Membership Committee.*** (Total to date—2.)
Broadhurst, Lee, Rogers, Ark.

CALIFORNIA

Roadhouse, C. L., ***Chairman, State Membership Committee.*** (Total to date—12.)
Denny, James M., San Francisco, Calif.
Kohler, Roland, Los Angeles, Calif.
Manning, Paul D. V., San Francisco, Calif. (nominated by Turnbow).

CONNECTICUT

Mann, A. I., ***Chairman, State Membership Committee.*** (Total to date—2.)
Atwood, Grover C., Storrs, Conn. (Student Affiliate) (by White).

DISTRICT OF COLUMBIA

LeFevre, P. E., ***Chairman, Membership Committee.*** (Total to date—2.)

GEORGIA

Bennett, F. W., ***Chairman, State Membership Committee.*** (Total to date—6.)
Frey, E. W., Douglas, Ga.
Hodgson, Harold B., Athens, Ga.

IDAHO

Fourt, D. L., ***Chairman, State Membership Committee.*** (Total to date—1.)
Wise, Arthur, Salmon, Idaho.

ILLINOIS

Kuhlman, A. F., ***Chairman, State Membership Committee.*** (Total to date—13.)
Eisenstein, Norman, Chicago, Ill. (by Ruehe).
Greene, Howard T., Genesee Depot, Wisc. (by Yapp).
Harshbarger, Kenneth E., Urbana, Ill.
Hemb, D. M., Chicago, Ill. (by Ruehe).
Ingle, J. D., Chicago, Ill. (by Parsons).
Keeffer, Kyle W., Aurora, Ill. (by Ruehe).
Kuhlman, A. F., Urbana, Ill.
Scott, E. C., Chicago, Ill. (by Parsons).
Edman, George J., Urbana, Ill. (Stud. Aff.) (by Ruehe).

INDIANA

Wilbur, J. W., ***Chairman, State Membership Committee.*** (Total to date—29.)
Epple, William F., Lafayette, Ind.
Gannon, E. A., W. Lafayette, Ind.
Logan, Paul S., Lafayette, Ind.

IOWA

Johnston, Floyd, ***Chairman, State Membership Committee.*** (Total to date—23.)
Anderson, Donald E., Ames, Iowa (by Iverson).

Baird, Phil, Lohrville, Iowa (by Hammer).
Berry, John C., Ames, Iowa.
Cannon, Robert Y., Ames, Iowa.
Conner, J. K., Mandan, N. Dak. (by Iverson).
Cromer, Paul C., Emmetsburg, Iowa.
Eaton, Hamilton, Ames, Iowa.
Emerson, John, Emmetsburg, Iowa.
Finch, Lindley, Dallas Center, Iowa.
Ingels, John W., Waverly, Iowa.
Jaekels, Dallas C., Ames, Iowa.
Knapp, Seaman A., Ames, Iowa (by Iverson).
Lynch, Arthur D., St. Louis, Mo.
Medin, Carl D., Mason City, Iowa.
Micheel, Vernon L., Ames, Iowa.
Thomas, Dr. B. H., Ames, Iowa.
Weaver, Russell C., Mason City, Iowa.
Willey, R. C., Mason City, Iowa.
Underbjerg, Gravers, Ames, Iowa.

KANSAS

Linn, J. W., *Chairman, State Membership Committee.* (Total to date—1.)

KENTUCKY

Ely, Fordyce, *Chairman, State Membership Committee.* (Total to date—4.)
Burnette, W. P., Mayfield, Ky.
Foster, J. D., Lexington, Ky.
Smathers, Jas. B., Lexington, Ky.
Wilson, Philip, Lexington, Ky.

LOUISIANA

Lush, R. H., *Chairman, State Membership Committee.* (Total to date—2.)
Fahrig, Chris G., Taft, La.

MARYLAND

England, C. W., *Chairman, State Membership Committee.* (Total to date—7.)

MASSACHUSETTS

Frandsen, J. H., *Chairman, State Membership Committee.* (Total to date—5.)
Smith, Albert W., Springfield, Mass.

MICHIGAN

Weaver, Earl, *Chairman, State Membership Committee.* (Total to date—30.)
Johnson, George K., East Lansing, Mich.

MINNESOTA

Macy, H., *Chairman, State Membership Committee.* (Total to date—25.)
Ahlstrand, Eric R., St. Paul, Minn.
Bergford, Harold L., Moorhead, Minn.
Brereton, John C., St. Paul, Minn.
Collis, Harold W., St. Paul, Minn.
Hogenson, Raymond B., St. Paul, Minn. (by Coulter).
Mattson, Walfrid T., Hibbing, Minn.
Shaw, Joseph C., St. Paul, Minn.

MISSISSIPPI

Herzer, F. H., *Chairman, State Membership Committee.* (Total to date—3.)
Mitchell, S. B., Uniontown, Ala.

MISSOURI

Herman, H. A., *Chairman, State Membership Committee.* (Total to date—9.)
Decker, Clinton W., Columbia, Mo. (Stud. Aff.).
Garrison, Earl R., Columbia, Mo.
Hall, W. C., Columbia, Mo.
Hunt, Edw. S., Tonganoxie, Kans.
Mixner, John P., Columbia, Mo.
Skinner, G. R., Bonne Terre, Mo.

MONTANA

Nelson, J. A., *Chairman, State Membership Committee.* (Total to date—9.)
Bates, Richard M., Great Falls, Mont.
Horkan, George, Bozeman, Mont.
Kopland, D. V., Huntley, Mont.

NEW HAMPSHIRE

Morrow, K. S., *Chairman, State Membership Committee.* (Total to date—1.)
Hall, Clyde N., Durham, N. H.

NEW YORK

Salisbury, G. W., *Chairman, State Membership Committee.* (Total to date—27.)
Albrectsen, Raymond, Ithaca, N. Y.
Bard, James M., Thornwood, N. Y.
Cairns, G. M., Ithaca, N. Y.
Doob, Hugo, Jr., Ithaca, N. Y. (by Holland).
Duck, Ira B., Jr., New York, N. Y.
Emasiri, Saluey, Ithaca, N. Y. (by Holland).
Gunsalus, Irwin C., Ithaca, N. Y. (by Holland).
Harrison, Edwin, Ithaca, N. Y.
Kahlenberg, Orme J., Bainbridge, N. Y. (by Noll).
Landsburg, Kenneth, New York, N. Y.
Lu, Louis Kao Hui, Ithaca, N. Y. (by Holland).
Robinson, L. W., Alfred, N. Y. (by Myers).
Santa Maria, Anthony A., Richmond Hill, N. Y. (by Herrington).
Stewart, Aubrey P., Ithaca, N. Y. (by Holland).
Botwinik, Leonard, Ithaca, N. Y. (Stud. Aff.).
Shapiro, Norman, Ithaca, N. Y. (Stud. Aff.).

OHIO

Salisbury, S. M., *Chairman, State Membership Committee.* (Total to date—45.)
Bishop, Robt. W., Greenville, O.
Boone, W. J., Marysville, O. (by Titus).
Grinnell, Cornelius, Yellow Springs, O.
Groves, Elden R., Spencer, Ind.
Haas, Norman F., Orrville, O.
Kahler, Karl M., Chagrin Falls, O.
Little, Ralph B., Princeton, N. J. (by Sutton).
Lohmeyer, Carl, Swedesboro, N. J.
Nadelin, Eugen Paul, Wooster, O.

Starbuck, Raymond R., Rio Grande, O.
Thompson, John C., New York, N. Y.
Wagner, Wm., Canal Fulton, O.
Wehr, Wade N., Youngstown, O.
Wisler, A. G., Sharon Center, O.
Babcock, Wayne, Toledo, Ohio (Stud. Aff.) (by Stoltz).
Summe, John J., Columbus, O. (Stud. Aff.) (by Stoltz).
Thomas, Elmer L., Columbus, O. (Stud. Aff.) (by Stoltz).

OREGON

Wilster, G. H., *Chairman, State Membership Committee.* (Total to date—4.)
Fletcher, Norman, Coquille, Ore.
Haag, J. R., Corvallis, Ore.
Steel, George F., Olympia, Wash.

PENNSYLVANIA

Dahle, C. D., *Chairman, State Membership Committee.* (Total to date—74.)
Anderson, Theodore Gustave, State College, Pa. (by Doan).
Brown, Irvan A., Lloydell, Pa.
Chico, Samuel, Morgantown, W. Va.
Corley, Robert T., Sandwich, Ill.
Dizikes, Jim L., State College, Pa.
Helms, A. LeRoy, Bethlehem, Pa.
Horrobin, Robert E., Arlington, N. J.
Johnston, H. K., State College, Pa.
Judson, Joseph B., Jr., Franklin, Pa.
Lotz, John H., Clifton, N. J.
Parthemore, John A., New Cumberland, Pa.
Sauers, Harry E., Harrisburg, Pa.
Schwartz, Charles, Pittsburgh, Pa.
Storch, Kenneth E., Columbia Cross Roads, Pa.
Ulrich, Edgar W., Penbrook, Pa.
Wintor, Dudley W., Glenside, Pa.
Yoder, R. L., Goldsboro, Md.
Zwicky, Arnold M., Berkshire Heights, Pa.
Andrews, George L., Philadelphia, Pa. (Stud. Aff.).
Brunner, J. Robert, Royers Ford, Pa. (Stud. Aff.).
Carber, Henry N., Boyertown, Pa. (Stud. Aff.).
Cober, Paul H., Garrett, Pa. (Stud. Aff.).
DeVincentis, Eugene R., State College, Pa. (Stud. Aff.).
Diehl, M. H., State College, Pa. (Stud. Aff.).
Dunmire, Ira N., McVeytown, Pa. (Stud. Aff.).
Ferro, Joseph C., State College, Pa. (Stud. Aff.).
Ginter, John D., Halifax, Pa. (Stud. Aff.).
Goodnoe, Raymond, Newtown, Pa. (Stud. Aff.).
Hanna, Roy M., State College, Pa. (Stud. Aff.).
Harter, Sherman H., Trucksville, Pa. (Stud. Aff.).
Herman, C. R., State College, Pa. (Stud. Aff.).
Hertel, Charles A., Pittsburgh, Pa. (Stud. Aff.).
Hubbell, Bruce, Erie, Pa. (Stud. Aff.).
Keen, Robert H., Willow Street, Pa. (Stud. Aff.).
Kohler, Casper, State College, Pa. (Stud. Aff.).

Lang, Julius F., Philadelphia, Pa. (Stud. Aff.).
Marklo, David L., Jr., State College, Pa. (Stud. Aff.).
McGrail, Gerald M., State College, Pa. (Stud. Aff.).
Melick, Larue T., State College, Pa. (Stud. Aff.).
Merry, Walter C., McKeesport, Pa. (Stud. Aff.).
Miller, Kenneth, Bethlehem, Pa. (Stud. Aff.).
Mowery, Albert S., State College, Pa. (Stud. Aff.).
Ramsey, W. Allen, State College, Pa. (Stud. Aff.).
Shellenberger, John L., Columbia, Pa. (Stud. Aff.).
Snee, E. L., Pittsburgh, Pa. (Stud. Aff.).
Torrance, Hugh, Jeannette, Pa. (Stud. Aff.).
Turner, Walton L., Pittsburgh, Pa. (Stud. Aff.).
Weber, Myer, Lake Ariel, Pa. (Stud. Aff.).
Wersto, Peter A., Boswell, Pa. (Stud. Aff.).
Wiggins, Robert, State College, Pa. (Stud. Aff.).

SOUTH CAROLINA

LaMaster, J. P., *Chairman, State Membership Committee.* (Total to date—12.)
Brannon, C. C., Clinton, S. C.
Caughman, D. H., Chester, S. C.
Cochran, J. W., Jr., Nashville, Tenn.
Faires, E. W., Columbia, S. C.
McGee, K. W., Charlotte, N. C.
McGee, R. H., Charleston, S. C.
Moss, J. R., Atlanta, Ga.
Norris, J. F., Miami, Fla.
Smith, C. T., Kinards, S. C.
Steer, R. L., Greenville, S. C.
Till, Dr. S. B., Asheville, N. C.

TENNESSEE

Harrison, T. B., *Chairman, State Membership Committee.* (Total to date—2.)
Hinton, S. A., Knoxville, Tenn.
Tretsven, Wayne, Knoxville, Tenn.

TEXAS

Renner, K. M., *Chairman, State Membership Committee.* (Total to date—12.)
Harris, Carl, Shreveport, La.
Heierman, Dan H., Portales, N. Mex.
Hill, James, Clovis, N. Mex.
Kilgore, J. P., Ardmore, Okla.
Nelson, Joseph J., Lubbock, Tex. (by Pederson).
Weddle, W. N., Albuquerque, N. Mex.
Bell, W. F., Lubbock, Tex. (Stud. Aff.).
Huffman, James, Lubbock, Tex. (Stud. Aff.).
Reast, Kenneth, Lubbock, Tex. (Stud. Aff.).
Scheid, Van, Lubbock, Tex. (Stud. Aff.).
Wilkowske, Howard H., Lubbock, Tex. (Stud. Aff.).
Wilson, George, Lubbock, Tex. (Stud. Aff.).

UTAH

Morris, A. J., *Chairman, State Membership Committee.* (Total to date—18.)
Cole, Elvin G., Salt Lake City, Utah (by Merrill).

Ekins, Ernest, Ogden, Utah.
Pyper, Glen G., Salt Lake City, Utah.
Stock, Orville, Ogden, Utah.
Harris, Marion, Logan, Utah (Stud. Aff.).
Hoskisson, William A., Logan, Utah (Stud. Aff.).
Stewart, Ellwood F., Logan, Utah (Stud. Aff.).

VERMONT

Ellenberger, H. B., *Chairman, State Membership Committee.* (Total to date—10.)
Bowen, Clark H., Bellows Falls, Vt.
Gilmore, John, St. Albans, Vt.
Ware, L. D., Richmond, Vt.
Whitworth, John, Burlington, Vt. (Stud. Aff.).

VIRGINIA

Connelly, R. G., *Chairman, State Membership Committee.* (Total to date—11.)
Cline, Stanley B., Richmond, Va.
Franklin, Norman L., Salem, Va.
Mallory, T. E., Bland, Va.
Miller, W. C., Roanoke, Va.
Wilson, I. B., Smithfield, Va.
Woods, E. C. C., Richmond, Va.
Young, Delmar J., Blacksburg, Va.

WASHINGTON

Bendixen, H. A., *Chairman, State Membership Committee.* (Total to date—24.)
Boyd, James Cecil, Spokane, Wash.
Eagles, Blythe, Vancouver, B. C., Canada (by Golding).
Forster, Hans, Issaquah, Wash.
Goodwin, Dwight, Hillyard Sta., Wash.
Johnson, Lister, Pullman, Wash.
Sturrock, Philip, Ft. Steilacoom, Wash.
Suckling, Sydney, Seattle, Wash.
Ticknor, E. L., Centralia, Wash.
Van Amburgh, John E., Colfax, Wash.

WEST VIRGINIA

Bowling, G. A., *Chairman, State Membership Committee.* (Total to date—2.)
Cook, Glen, Morgantown, W. Va.
Putnam, Dexter N., Morgantown, W. Va.

WISCONSIN

Weckel, K. G., *Chairman, State Membership Committee.* (Total to date—26.)
Bowman, Duane, Madison, Wisc.
Carlson, E. E., Elroy, Wisc.
Fick, Harold F., Madison, Wisc.
Knoll, Roy P., Milwaukee, Wisc.
Paulson, Emil, Champaign, Ill.
Rasmussen, Harold L., Madison, Wisc.
Schmiege, Clement C., Oostburg, Wisc.
Stellman, Clarence, New Albany, Ind.
Stern, Robert M., Madison, Wisc.

WYOMING

Willard, H. S., *Chairman, State Membership Committee.* (Total to date—3.)
Fellhauer, Tony, Laramie, Wyo.
Wray, C. S., Afton, Wyo.

CANADA (British Columbia)

Rive, Henry, *Chairman, Membership Committee.* (Total to date—4.)

CANADA (Eastern Provinces)

Raithby, F. E., *Chairman, Membership Committee.* (Total to date—2.)

CANADA (Prairie Provinces)

Potts, A. E., *Chairman, Membership Committee.* (Total to date—3.)
Ketcheson, Earl, Winnipeg, Man., Can.
Killick, C. H. P., Winnipeg, Man., Can.
Nesbitt, W. S., St. Lazare, Man., Can.

CANADA

Brown, R. W., *Chairman, Membership Committee.* (Total to date—1.)

MISCELLANEOUS

Crow, Carl D., Holliday's Cove, W. Va.
Ketcham, W. J., Columbus, O.
Shepard, Sidney, Birmingham, Ala.
Gamble, J. A., Washington, D. C.

JOURNAL OF DAIRY SCIENCE

VOLUME XXII JUNE, 1939 NUMBER 6

PROGRAM

THIRTY-FOURTH ANNUAL MEETING
THE AMERICAN DAIRY SCIENCE ASSOCIATION

STATE COLLEGE OF WASHINGTON
PULLMAN, WASHINGTON

AND

UNIVERSITY OF IDAHO
MOSCOW, IDAHO

JUNE 27-30, 1939

PROGRAM COMMITTEE

P. A. DOWNS, University of Nebraska
H. W. CAVE, Kansas State College
S. J. BROWNELL, Cornell University
H. P. DAVIS (Advisory member), University of Nebraska
T. S. SUTTON (Advisory member), Ohio State University
E. V. ELLINGTON (Chairman), State College of Washington

AMERICAN DAIRY SCIENCE ASSOCIATION

The Thirty-Fourth Annual Meeting

Pullman, Washington and Moscow, Idaho

June 27–30, 1939

GENERAL PROGRAM

Monday, June 26

1:30 P.M.–5:00 P.M. General Registration and Room Registration, Davis S. Troy Hall, Pullman, Washington, or Willis Sweet Hall, Moscow, Idaho.

8:00 P.M. Board of Directors meeting, Troy Hall, Pullman, Washington.

Tuesday, June 27

8:00 A.M.–12:00 NOON General Registration and Room Registration, David S. Troy Hall, Pullman, Washington, or Willis Sweet Hall, Moscow, Idaho.

9:30 A.M.–12:00 NOON MANUFACTURING SECTION.

Dairy Products Judging Conference for coaches and instructors, Room 114, Troy Hall, Pullman, Washington. Scoring practice for ice cream and cheese.

1:30 P.M.–3:30 P.M. OPENING SESSION. Auditorium, Administration Building, University of Idaho, Moscow, Idaho, Earl Weaver, Presiding.

General Session. Addresses of Welcome by H. C. Dale, President, University of Idaho, and E. O. Holland, President, State College of Washington.

Response and President's address by Earl Weaver, President, American Dairy Science Association.

Introduction of past presidents, officers and directors of the American Dairy Science Association.

Objectives of Dairy Husbandry Teaching in Secondary Schools. H. E. Lattig, Assistant Dean of Agriculture, University of Idaho.

Adult Education in Dairy Husbandry. E. V. Ellington, Vice Dean and Director, College of Agriculture and Experiment Station, State College of Washington.

3:30 P.M. Committee meetings. University of Idaho. Rooms to be assigned at registration office.

8:00 P.M. SOCIAL HOUR. Women's Gymnasium, State College of Washington.

Wednesday, June 28

8:30 A.M.–9:30 A.M. COMMITTEE MEETINGS. University of Idaho. Rooms to be assigned at registration office.

	Inspection of extension exhibits. Room 101 and 113, Wilson Hall, State College of Washington.
9:30 A. M.–12:00 NOON	SECTIONAL MEETINGS. Manufacturing Section, Science Hall, Room 110, University of Idaho. Production Section, U. C. B. Room 101, University of Idaho. Extension Section, U. C. B. Room 102, University of Idaho.
12:00 NOON	COMPLIMENTARY LUNCHEON FOR MEMBERS—Student Union Building, University of Idaho.
1:30 P. M.–3:30 P. M.	SECTIONAL MEETINGS. University of Idaho. Manufacturing Section, Science Hall, Room 110, University of Idaho. Production Section, U. C. B. Room 101, University of Idaho. Extension Section, U. C. B. Room 102, University of Idaho.
3:30 P. M.	COMMITTEE MEETINGS. Rooms to be assigned at registration office.
8:00 P. M.	ANNUAL ASSOCIATION RECEPTION. Willis Sweet Hall, University of Idaho.

Thursday, June 29

8:30 A. M.–9:30 A. M.	COMMITTEE MEETINGS. State College of Washington. Rooms to be assigned at registration office.
9:30 A. M.–12:00 NOON	SECTIONAL MEETINGS. Manufacturing Section, New Science Hall, Room 207, State College of Washington. Production Section, James Wilson Hall, Room 13, State College of Washington. Extension Section, James Wilson Hall, Room 112, State College of Washington.
	Group picture at Field House, State College of Washington.
	Complimentary Picnic Lunch, Field House, State College of Washington.
1:30 P. M.–3:30 P. M.	SECTIONAL MEETINGS. Manufacturing Section, New Science Hall, Room 207, State College of Washington. Production Section, James Wilson Hall, Room 13, State College of Washington. Extension Section, James Wilson Hall, Room 112, State College of Washington.
3:30 P. M.	SECTIONAL BUSINESS MEETINGS. Same rooms as sectional meetings above.
6:30 P. M.	ANNUAL ASSOCIATION BANQUET. Washington Hotel, Pullman, Washington. Presentation of Borden Awards.

Friday, June 30

8:30 A. M.–9:30 A. M.	Sectional Business Meetings. Manufacturing Section, New Science Hall, Room 207, State College of Washington. Production Section, James Wilson Hall, Room 13, State College of Washington. Extension Section, James Wilson Hall, Room 112, State College of Washington.
9:30 A. M.–12:00 NOON	General Session, Earl Weaver, Presiding. Room 207, New Science Hall, State College of Washington. Reports to the general meeting of Board of Directors, of Sections, and General Association Committees for discussion and action.

SECTION PROGRAMS

MANUFACTURING SECTION

Tuesday A. M., June 27, 9:30–12:00
Room 114, David S. Troy Hall,
State College of Washington

Dairy Products Judging Conference for Coaches and Instructors
Scoring practice for cheese and ice cream

H. A. Bendixen and D. R. Theophilus, in charge.

Wednesday A. M., June 28, 9:30–12:00
Science Hall 110
University of Idaho

P. A. Downs, *Chairman*

Market Milk

M1—The use of sodium metaphosphate for the preparation of soft-curd milk. Charles Schwartz, K. K. Jones, T. W. Mack, and R. W. Vance, Hall Laboratories, Inc. and Northwestern University Medical School.

M2—Effect of certain sodium salts upon the curd tension of milk. P. H. Tracy and W. J. Corbett, University of Illinois.

M3—Further studies of "curd surface" in relation to the digestibility of milk. Leslie A. Chambers, Irving J. Wolman and A. P. Hands, University of Pennsylvania.

M4—Photochemical study of the irradiation process of producing vitamin D in milk. M. J. Dorcas, National Carbon Co., Inc., Cleveland, Ohio.

M5—Effect of concentrating milk in the vacuum pan on the development of oxidized flavors. W. J. Corbett and P. H. Tracy, University of Illinois.

M6—Rates of corrosion in milk of certain "white-metal" copper alloys and their influence on the flavor of milk. C. L. Roadhouse and J. L. Henderson, University of California.

M7—The rate of destruction of vitamin C in milk as an index of the solubility of copper from copper-nickel alloys. J. L. Henderson and C. L. Roadhouse, University of California.

M8—The effect of winter and summer rations, and cod-liver oil on the oxidized flavors and vitamin C in milk. E. S. Guthrie, Cornell University.

M9—Studies in rancid milk. Relation of surface tension of rancid milk to its acid coagulation. N. P. Tarassuk and F. R. Smith, University of California.

M10—A study of some factors affecting the viscosity of market cream. F. M. Skelton and H. B. Ellenberger, Vermont Agricultural Experiment Station.

PRODUCTION SECTION

Wednesday A. M., June 28, 9:30–12:00
U. C. B., Room 101
University of Idaho

H. W. CAVE, *Chairman*

Milk Secretion and Physiology and Udder Diseases

P1—The lactation-promoting properties of proteins as measured with rats. J. R. Haag and L. D. Wright, Oregon Agricultural Experiment Station.

P2—Recent advances in the endocrinology of milk secretion. C. W. Turner, Missouri Agricultural Experiment Station.

P3—Some factors affecting the reliability of arterio-venus differences in a study of blood precursors of milk. W. E. Petersen and W. L. Boyd, University of Minnesota.

P4—The respiratory quotients of the intact and perfused mammary glands of cows. J. C. Shaw, University of Minnesota.

P5—Perfusion of the excised mammary gland as a method of studying milk secretion. W. E. Petersen, J. C. Shaw, and M. B. Visscher, University of Minnesota.

P6—The effect of ultraviolet radiation on milk production. W. E. Krauss, C. F. Monroe, C. C. Hayden, and B. H. Edgington, Ohio Agricultural Experiment Station.

P7—Studies on milk and fat production of dairy cows and goats as influenced by thyroxine. N. P. Ralston, C. W. Turner and A. C. Ragsdale, University of Missouri.

P8—Two methods of feeding soybean fat to cows and their effect on milk and butterfat production and on the nature of the butterfat. Kenneth Williams, C. Y. Cannon and Dwight Espe, Iowa State College.

P9—The effect of sulfanilamide therapy upon the catalase activity of the blood and milk. A preliminary report. P. G. Miller and G. W. Anderson, South Carolina Agricultural Experiment Station.

P10—The effect of ultraviolet radiation of cows on their agglutinin titer for Bangs disease. B. H. Edgington, W. E. Krauss, C. F. Monroe and C. C. Hayden, Ohio Agricultural Experiment Station.

P11—A new routine extending the usefulness of the Hotis test for mastitis. J. Frank Cone, Bureau of Dairy Industry, U.S.D.A.

P12—The classification of the streptococci of bovine mastitis. R. B. Little, The Rockefeller Institute for Medical Research, Princeton, N. J. and F. C. Minett, Royal Veterinary College, Camden Town, London.

EXTENSION SECTION

Wednesday, A. M., June 28, 9:30–12:00
U. C. B., Room 102
University of Idaho
S. J. BROWNELL, *Chairman*

E1—Community dairy cattle breeding plans. Sire Committee, J. F. Kendrick, U. S. Bureau of Dairy Industry, *Chairman.*

E2—Bull studs in the community dairy cattle breeding program. Ivan H. Loughary, University of Idaho.

E3—Extension Farm-Security Administration bull associations in Utah. Lyman H. Rich, Utah State College.

E4—The progress and prospects of organized artificial insemination of dairy cattle. E. J. Perry, New Jersey College of Agriculture.

E5—Utilization of proved sire records and herd analysis data. Sire Committee, J. F. Kendrick, U. S. Bureau of Dairy Industry.

E6—Utilization on State and County basis. Warren Gifford, University of Missouri.

E7—Utilization in the individual dairy herd. C. F. Rowe, University of Arizona.

E8—The influence of disease in dairy cattle breeding programs. George E. Taylor, New Jersey Agricultural College.

MANUFACTURING SECTION

Wednesday P. M., June 28, 1:30–3:30
Science Hall, Room 110
University of Idaho
P. A. DOWNS, *Chairman*

Ice Cream

M11—A comparative evaluation of an ice cream supply as it reaches the consumer. L. K. Crowe and P. A. Downs, University of Nebraska.

M12—Measuring the quality of ice cream. W. H. Martin, F. E. Nelson and W. J. Caulfield, Kansas Agricultural Experiment Station.

M13—Consumer preference for individual chocolate varieties. P. S. Lucas and I. A. Gould, Michigan State College.

M14—The use of the petrographic microscope in studying the different types of lactose crystals as they occur in sandy ice cream. W. S. Arbuckle, C. W. Decker and W. H. E. Reid, Missouri Agricultural Experiment Station.

M15—The use of motion pictures in studying the influence of several factors upon the stability of ice cream and sherbets. W. H. E. Reid, W. S. Arbuckle and C. W. Decker, Missouri Agricultural Experiment Station.

M16—Microscopic technique for studying fat globules in dairy products and other oil in water emulsions. W. C. Cole, University of California.

M17—The effect of the new standard agar on the plate counts of dairy products. F. E. Nelson, Kansas State College.

M18—The development on new standard milk agar of certain organisms of importance to the dairy industry. V. D. Foltz and F. E. Nelson, Kansas Agricultural Experiment Station.

M19—Regulatory legislation and its relationship to research in manufactured dairy products. Charles S. Trimble, U. S. Bureau of Dairy Industry.

PRODUCTION SECTION

Wednesday P. M., June 28, 1:30–3:30
U. C. B., Room 101
University of Idaho
A. H. KUHLMAN, *Chairman*

P13—Results of a long time feeding experiment on bone meal vs. no bone meal in dairy ration consisting of timothy hay, corn silage, and concentrates. S. I. Bechdel, P. S. Williams, A. A. Borland, and J. F. Shigley, Pennsylvania State College.

P14—Making and feeding air dried hay to dairy heifers. C. E. Wylie and S. A. Hinton, University of Tennessee; J. W. Weaver, Tennessee Valley Authority.

P15—The nutritive value of an alfalfa hay ration compared with a mixed grass and legume hay and grass silage ration for dairy cattle. R. E. Hodgson, J. C. Knott, State College of Washington and Bureau of Dairy Industry.

P16—Studies with alfalfa hay for milk production. I. R. Jones and J. R. Haag, Oregon Agricultural Experiment Station.

P17—Comparative yields of Louisiana feed and pasture crops. R. H. Lush, Louisiana Agricultural Experiment Station.

P18—Pasture yields as measured by clip plots and by grazing dairy cows. P. M. Brandt and H. P. Ewalt, Oregon Agricultural Experiment Station.

P19—The chemical composition and nutrient yield of pasture grass grazed at successive intervals during the season. R. E. Hodgson and J. C. Knott, Bureau of Dairy Industry and State College of Washington.

P20—Some relations of the roughage intake to the composition of milk. E. B. Powell, Ralston Purina Company, St. Louis, Missouri.

P21—The influence of certain rations and management practices on the rate of growth and production of Holstein-Friesian heifers. H. A. Herman and A. C. Ragsdale, University of Missouri.

EXTENSION SECTION

Wednesday P. M., June 28, 1:30–4:30
U. C. B., Room 102
University of Idaho
S. J. BROWNELL, *Chairman*

E9—Should we unify the extension program? C. L. Blackman, Ohio State University.

E10—Michigan dairy planning activities. A. C. Baltzer, Michigan State College.

Extension Exhibits, Rooms 101 and 113
Wilson Hall, State College of Washington

Exhibits Committee Report. E. G. Scheidenhelm, Michigan State College. (Inspection and discussion of exhibits.)

MANUFACTURING SECTION

Thursday A. M., June 29, 9:30–12:00
New Science Hall, Room 207
State College of Washington
P. A. DOWNS, *Chairman*

Butter

M20—Studies with the rollerless churn. W. B. Combs and S. T. Coulter, University of Minnesota.

M21—The effect of the ration on butter fat constants. A. H. Kuhlman and W. D. Gallup, Oklahoma A. & M. College.

M22—Some observations on the iodine, Reichert-Meissl and Polenske Values of milk fat produced during the winter in three sections of Oregon. R. E. Stout and G. H. Wilster, Oregon State College.

M23—A preliminary report on investigations to overcome crumbly and sticky texture in butter made during the winter in the irrigated sections of eastern and southern Oregon. G. H. Wilster, R. W. Stein and R. E. Stout, Oregon State College.

M24—Copper in butter. B. E. Horrall and W. F. Epple, Agricultural Experiment Station, Purdue University.

M25—Acid values and acid ratios as related to the keeping quality of salted butter. H. A. Bendixen, Washington Agricultural Experiment Station.

M26—The influence of various factors on the reliability of the room temperature holding test for butter quality. D. H. Jacobsen, South Dakota Agricultural Experiment Station.

M27—Laboratory equipment for high temperature pasteurization studies. T. S. Coulter and W. B. Combs, University of Minnesota.

M28—Correlation between grades on scores and grades on criticisms in the judging of dairy products. G. M. Trout, Michigan State College.

PRODUCTION SECTION

Thursday A. M., June 29, 9:30–12:00
Room 13, Wilson Hall
State College of Washington
I. W. RUPEL, *Chairman*

Nutrition

P22—Vitamin A and carotene in relation to growth and well being of heifers. R. E. Ward and S. I. Bechdel, Pennsylvania State College.

P23—Carotene changes in natural cured, artificially dried and ensiled roughages. O. M. Camburn, H. B. Ellenberger, and G. C. Crooks, Vermont Agricultural Experiment Station.

P24—Some effects of feeding vitamin A and carotene to cows, on the composition of their milk and blood. C. H. Whitnah, W. J. Peterson, H.

W. Cave and F. W. Atkeson, Kansas Agricultural Experiment Station.

P25—The effect of feeding vitamin A and carotene to cows on the flavor of their milk. H. W. Cave, W. H. Martin and G. H. Beck, Kansas Agricultural Experiment Station.

P26—Anatomical and physiological changes in the pituitary gland in vitamin A deficiency. T. S. Sutton, Ohio State University.

P27—A field study of the influence of restricted winter rations on the blood calcium, phosphorus and carotene of dairy cattle. W. H. Riddell, F. W. Atkeson, W. J. Peterson and W. W. Thompson, Kansas Agricultural Experiment Station.

P28—Rickets in calves, a regional problem. A. H. Kuhlman and W. D. Gallup, Oklahoma A. and M. College.

P29—Further studies on the vitamin D deficiency of mature dairy cows. G. C. Wallis, South Dakota Experiment Station.

P30—Vitamin E studies with male rats. A. O. Shaw, Ella Woods, and J. O. English, University of Idaho.

P31—Are urea and other simple nitrogen compounds useful in dairy cattle feeding? E. B. Hart, G. Bohstedt, H. J. Deobald, and M. I. Wegner, University of Wisconsin.

P32—The relation of the rate of disappearance of milk curd from the calf's abomasum to the apparent digestibility of its protein. H. C. Dickey, Dwight Espe and C. Y. Cannon, Iowa State College.

P33—Factors affecting the reactions of the esophageal groove of dairy calves. George H. Wise, South Carolina Agricultural Experiment Station.

EXTENSION SECTION

Thursday A. M., June 29, 9:30–12:00
Wilson Hall, 112
State College of Washington
S. J. BROWNELL, *Chairman*

E11—Testing Committee Report. Glen Vergeront, University of Wisconsin, *Chairman.*

E12—Annual D. H. I. A. state reports or summaries, their preparation and use. Roger W. Morse, Oregon State College.

E13—D. H. I. A. methods and results in California. G. E. Gordon, University of California.

E14—Feeding Committee Report. G. G. Gibson, Texas A. and M. College, *Chairman.*

E15—The analysis and use of D. H. I. A. feed records. C. G. Cushman, Clemson Agricultural College.

E16—Progress of demonstrations with grass and legume silage. D. M. Seath and James W. Linn, Kansas State College.

E17—Pasture improvement as a project in the state dairy extension program. Otto J. Hill, State College of Washington.

MANUFACTURING SECTION

Thursday P. M., June 29, 1:30–3:30
New Science Hall, Room 207
State College of Washington
P. A. DOWNS, *Chairman*

Cheese

M29—Standards for rating cheddar cheese. J. C. Marquardt, New York Agricultural Experiment Station (Geneva).

M30—Observations on the growth responses of *S. lactis* in mastitis milk. C. C. Prouty, Washington Agricultural Experiment Station.

M31—Potentiometric studies with resazurin and methylene blue in milk. C. K. Johns and R. K. Howson, Department of Agriculture, Ottawa, Canada.

M32—Lactic acid streptococci of cheese. Blythe A. Eagles and Olga Okulitch, University of British Columbia, Vancouver, Canada.

M33—Application of the smear culture technique to the examination of cheddar cheese. C. B. Lane and B. W. Hammer, Iowa State College.

M34—Relationship of fat hydrolysis to flavor development in cheddar cheese. C. B. Lane and B. W. Hammer, Iowa State College.

M35—Some factors affecting the moisture content of Swiss cheese and their effects upon quality and yield. George P. Sanders and Robert R. Farrar, U. S. Bureau of Dairy Industry.

M36—Further studies on the gas requirements of three strains of blue mold (*Penicillium Roqueforti* group) isolated from cheese. N. S. Golding, Washington Agricultural Experiment Station.

M37—The lipolytic and proteolytic activities of various penicillia of importance in the ripening of Blue Cheese. C. Jensen, North Dakota Agricultural Experiment Station.

PRODUCTION SECTION

Thursday P. M., June 29, 1:30–3:30
Room 13, Wilson Hall
State College of Washington

H. W. CAVE, *Chairman*

P34—Artificial insemination of dairy cows. H. A. Herman and A. C. Ragsdale, University of Missouri.

P35—The inheritance of the condition for a white head in Holstein-Friesian cattle. Lester O. Gilmore, Wm. E. Petersen and J. B. Fitch, University of Minnesota.

P36—The inheritance of fertility in dairy cattle. Arless Spielman and I. R. Jones, Oregon State College.

P37—Analyses of dairy herd breeding records. S. W. Mead and W. M. Regan, University of California.

P38—Increasing production in institutional herds. A. L. Beam, C. S. McLearen and A. A. Borland, Pennsylvania State College.

P39—The use of various oils and fats for calf feeding. T. W. Gullickson and F. C. Fountaine, University of Minnesota.

P40—The influence of fineness of grinding on the digestion coefficients of oats for dairy cows. G. C. Wallis, T. M. Olson and T. A. Evans, South Dakota Agricultural Experiment Station.

P41—The effect of changes in environmental temperature on water consumption of producing dairy cows. W. M. Regan and S. W. Mead, University of California.

EXTENSION SECTION

Thursday P. M., June 29, 1:30–3:30
Wilson Hall, 112
State College of Washington
S. J. BROWNELL, *Chairman*

E18—Report of Quality Improvement Committee. C. J. Babcock, U. S. Bureau of Dairy Industry, *Chairman.*

E19—Growth of Pennsylvania 4-H Club Calves. J. C. Nageotte, Pennsylvania State College.

E20—The contribution of 4-H Dairy Clubs to the general dairy extension program. J. O. Tretsven, Montana State College.

E21—Report of Type Classification Committee. A. C. Baltzer, Michigan State College, *Chairman.*

ABSTRACTS OF PAPERS PRESENTED AT ANNUAL MEETING

MANUFACTURING SECTION

M1. The Use of Sodium Metaphosphate for the Preparation of Soft-curd Milk.

Charles Schwartz, K. K. Jones, T. W. Mack, and R. W. Vance, Hall Laboratories, Inc., and Northwestern University Medical School Department of Physiology and Pharmacology.

The fact that glassy sodium metaphosphate reacts with calcium salts in aqueous solution so as to form a highly soluble, stable complex, from which the calcium cannot be precipitated by the usual reagents, has led to widespread industrial use of this heretofore unusual compound. Its characteristic property encouraged an inquiry into the possibility of using it as a means of preparing soft-curd milk.

A comprehensive investigation showed that the addition of an average of 0.04 gm. of sodium metaphosphate to 100 ml. of milk, either raw or pasteurized, was sufficient to yield a product forming no curd under the usual conditions of test. Somewhat greater quantities of metaphosphate, about 0.075 gm. for each 100 ml. of milk, were required to maintain a curd-free milk through a heating process. This may be attributed to a slight reversion of the metaphosphate to orthophosphate under the influence of heat.

By increasing the amount of metaphosphate, additional calcium could be incorporated in milk and zero curd tension maintained. The amount of metaphosphate required to soften milk in the presence of added calcium was roughly 4.8 times as much as the calcium added plus an amount necessary to soften the milk itself.

Digestion experiments "in vitro" and "in vivo" on rats and dogs indicated that the metaphosphate-softened milk is more efficiently digested than the untreated milk.

The preparation of soft-curd milk with metaphosphate is easily carried out, requires no special or new equipment, and in the amounts used affects neither the taste nor appearance of the milk.

M2. Effect of Certain Sodium Salts Upon the Curd Tension of Milk.

P. H. Tracy and W. J. Corbett, University of Illinois.

The addition of sodium citrate, tetra sodium pyrophosphate and sodium alginate have been found to lower the curd tension of milk. Sodium citrate and tetra sodium pyrophosphate are the most effective in this respect. By the addition of 0.15% tetra sodium pyrophosphate or 0.20% sodium citrate it was possible to lower the curd tension to 0, using the tentative standard method of the American Dairy Science Association. The reduction in curd tension was greater when the salts were added to the cold milk after pasteurization than when added before heating. A noticeable but not objectionable salty flavor results from the addition of the above indicated amounts of the sodium salts. It is thought that this method of producing soft curd milk may prove particularly useful for the small milk distributor limited in plant facilities.

M3. Further Studies of "Curd Surface" in Relation to the Digestibility of Milk.

Leslie A. Chambers, Irving J. Wolman, and A. P. Hands, Eldridge Reeves Johnson Foundation and the Department of Pediatrics, University of Pennsylvania.

The apparatus and technique for estimation of "curd surface" described in a previous communication, have been modified with three principal objects in view:

1. To provide greater reproducibility of individual measurements.
2. To make the test practicable for routine examination of milk and its modifications.
3. To make the conditions under which the coagulations are carried out approximate, as closely as possible, those encountered in the normal infant's stomach.

The modified procedure involves coagulation of the milk sample at pH 4.8. While the exact value was finally selected for pragmatic reasons, it lies in a range (4.5-5.5) indicated by measurements of pH of stomach contents, and by analysis of extensive published data. pH values lower than 4.5 permit more or less proteolysis which interferes with the measurement. The surface numbers depend on the final pH of the mixture and are not dependent on the rate at which the coagulant is added. Comparison of surface numbers derived from two different types of milk at pH values from 6.5 to 4.0 shows that the ratio of total surfaces is essentially constant over the entire range although there is a progressive increase in number with increase in acid concentration. This being true there seems no valid reason for coagulating the test samples at any other pH level than that encountered in the infant stomach.

Since the value of any *in vitro* test of digestibility can be determined only by correlation with actual feeding experience, we have fed a group of infants over a period of more than a year under carefully controlled conditions on milk indicated by the "surface number test" to be theoretically as digestible as milk boiled five minutes. Without exception this trial has been successful. We therefore believe that the curd surface area is an adequate index of the relative digestibility of milk in the infant's stomach. The results also demonstrate that at least one commercially processed milk (sonic homogenized) is improved in digestibility by the processing to such an extent that it can be fed without further modification to premature and new-born infants.

M4. Photochemical Study of the Irradiation Process of Producing Vitamin D in Milk. M. J. DORCAS, National Carbon Company, Inc., Cleveland, Ohio.

This paper will discuss the consideration of the mechanics of irradiating milk from the standpoint of the Grotthuss-Draper theory of photochemistry. This theory states that only the light absorbed by the reactants can be photochemically active. In milk irradiation ultra violet produced by alternating current sources may be intermittent in character, for instance the light may be generated and extinguished 120 times a second on the common 60 cycle current. Activatable sterols may be optically shielded during these brief periods of light emission. A light source operating on three electrical phases has been developed to test this theory. This type of light source emits light continuously. When applied to the irradiation of milk it is found to give higher Vitamin D potencies than previously were commercially obtainable.

M5. Effect of Concentrating Milk in the Vacuum Pan on the Development of Oxidized Flavors. W. J. CORBETT AND P. H. TRACY, University of Illinois.

Condensing milk under vacuum to a concentration of 2–1 was found to retard the development of oxidized flavors. Three parts per million of copper was added before or after condensing. Condensed whole milk diluted back to normal concentration with water or the distillate collected did not develop the flavor on addition of three parts per million of copper.

It was also observed that condensed milk diluted back to its original concentration had a curd tension of approximately a half of the original milk as determined by the Hill curd test. Other cases of a correlation between curd tension reduction and lessened tendencies toward oxidation flavors have been observed; for example, homogenization, agitation, addition of certain enzymes, high heat treatment, and addition of sodium salts. The problem of oxidized flavors in dairy products is being studied from this angle.

M6. Rates of Corrosion in Milk of Certain "White-Metal" Copper Alloys and Their Influence on the Flavor of Milk. C. L. ROADHOUSE AND J. L. HENDERSON, University of California.

Copper-nickel alloys have been used in the dairy industry for many years and those previously studied have been found to cause oxidized flavor in milk.

The modern trend has been to use corrosion-resistant metals in dairy equipment to avoid as far as possible the development of the oxidized flavor in diary products. Technical difficulties have been encountered in the fabrication from stainless steel of certain dairy equipment, such as fittings, valves and bearings.

A comparison was made of the corrosion of certain copper-nickel alloys in milk during the pasteurization process. The procedure used for studying the rates of corrosion was that previously described.[1] The alloys studied contained from 62 to 66.5 per cent copper and from 20 to 31 per cent nickel with smaller amounts of tin, lead, zinc, chromium, iron and manganese added to some of them.

The results show that when 3 to 8 per cent of tin and 3 to 4 per cent of zinc are included in the composition of copper-nickel alloys, the corrosion rates in milk are reduced and the development of oxidized flavor is delayed.

The data appear to indicate that tin and zinc, when included in the composition of the copper-nickel alloys, inhibit the solubility of the copper by milk.

Several samples showing differences in solubility of copper were polished, etched, and photomicrographed in an attempt to correlate the resistance to attack with the presence of secondary phases in the grain structure.

M7. The Rate of Destruction of Vitamin C in Milk as an Index of the Solubility of Copper from Copper-Nickel Alloys.

J. L. HENDERSON AND C. L. ROADHOUSE, University of California.

A common method for determining the rate of corrosion of metals by milk is to agitate the metals in milk during the pasteurizing process and determine the weight losses. The results of such determinations, in which a total of twenty copper-nickel alloys were tested, showed that this amount of exposure of the alloys to milk, in most cases caused sufficient solution of copper to develop oxidized flavor, except in the least susceptible milks. Milks so treated showed that most of the vitamin C was destroyed during pasteurization.

Since copper-nickel alloys are often used for fittings and valves, cast alloys were exposed to milk for brief periods to simulate commercial operation. The extent of vitamin C destruction when compared to that of control samples of the same milk, was used to indicate the amount of copper contamination. Copper, in the presence of oxygen, is a specific catalyst for the oxidation of vitamin C. The other important elements in the alloys, *i.e.*, zinc, lead and nickel, were found not to influence the rate of destruction of vitamin C.

The milks used were produced in such a manner that exposure to sunlight and metal contamination were avoided. Vitamin C was determined by titration of trichloracetic acid-metaphosphoric acid serum with sodium 2–6 dichloro-benzenone idophenol.

Minute amounts of copper in solution, such as resulted from dipping the copper alloys into milk 10, 20 and 100 times, could be detected from the rate of destruction of vitamin C. This method may be used to evaluate the copper contamination of milk by copper containing alloys exposed to milk for various periods at different temperatures.

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[1] Hilgardia *5*, 225–253, 1931.

M8. The Effect of Winter and Summer Rations, and Cod-Liver Oil on the Oxidized Flavors and Vitamin C in Milk. E. S. GUTHRIE, Cornell University.

The studies on the winter and summer rations extended over a period of one year and in most cases from one lactation period to another. The cows were fed the usual grains and hays as well as silage. Twelve cows from one herd receiving only corn silage, along with the usual amounts of grains and hays, were in the experiment and nine cows from another herd of Cornell University were studied. These cows received molasses-hay silage, phosphoric-acid-hay silage, and corn silage as a part of their roughage.

The pasteurized samples of milk were scored on the third and seventh days. The results were recorded in the same way as the oxidized flavors have been reported by a number of investigators. Vitamin C was determined by titration according to Sharp.

The four cows on the cod-liver oil experiment were fed by drenching, up to 400 ml. at a single feeding once each day.

SUMMARY

1. There was little difference in the oxidized flavors in the milk between the winter and the summer feedings. When the milk was three days old the oxidized score in the summer was .99 and in the winter it was .76. The seven-day reading was 1.45 and 1.49 respectively.

2. The grand averages of the milligrams per liter of reduced vitamin C in the fresh milk was practically the same, for the summer analyses showed a figure of 22.19, and the winter data were 22.21.

3. The milks of all four of the cows that were fed cod-liver oil were free from the oxidized and other off flavors at the beginning of the study. As the experiment progressed the fresh milk lost its characteristic flavors, and on standing a goaty flavor appeared in the milk of cows number 1, 2, and 3, while the milk from cow number 4 became very oxidized.

4. The following figures show the increases in reduced vitamin C in the milk from the check level when no cod-liver oil was fed, to the average of the three high readings when the amount of the oil fed was at high level: Cow No. 1, 111 per cent; cow No. 2, 31.3 per cent; cow No. 3, 48.1 per cent; and cow No. 4, 38.3 per cent.

M9. Studies on Rancid Milk. Relation of Surface Tension of Rancid Milk to Its Acid Coagulation. N. P. TARASSUK AND F. R. SMITH, University of California.

The partial inhibition of the growth of bacteria in rancid milk, particularly that of *Streptococcus lactis* with resultant delayed and abnormal (absence of regular firm clot) acid coagulation of milk has been reported by

Koestler, Roadhouse and Lörtscher (1928). The above authors gave no explanation for this phenomenon observed by them.

The data from the studies of Tarassuk and Palmer, Palmer and Tarassuk (in press), on physical and chemical properties of the adsorption "membrane" around the fat globules in cream indicated definitely that rancid milk must have significantly lower surface tension than that of non-rancid milk.

In the present studies on natural rancid milk it has been found that within certain limits there is a direct relationship between degree of rancidity of milk and its surface tension. As the hydrolytic rancidity develops the surface tension of milk decreases gradually from normal value of 50–52 dynes to 37–38 dynes. In fact, in our opinion the change in surface tension is the most profound change that differentiates the rancid milk from non-rancid milk and it has been used by one of us as a tool for general study of lipolytic activity of milk in preference to other methods hitherto employed for that purpose. The lowering of surface tension is due of course to fatty acids set free by hydrolysis.

The evidence was obtained that the phenomenon of delayed acid coagulation of rancid milk is due entirely to its comparatively low surface tension. When surface tension of milk is lowered by other means than the development of hydrolytic rancidity, such as by the addition of diglycol laurate or certain fatty acids, a similar phenomenon takes place. It is well known that surface tension is an important factor in growth of organisms and the surface tension of media in the neighborhood of 35 dynes is a critical one for the growth of *Streptococcus lactis.*

All samples of non-sterilized and sterilized rancid milk inoculated with *Streptococcus lactis* have shown the delayed growth. The important feature brought out in this connection is that when sterilized rancid milk is inoculated with *Streptococcus lactis* and set at optimum temperature, there is a somewhat longer than normal lag period. Furthermore, the growth during the logarithmic growth period is considerably slower (much longer generation time) than that of the normal milk. A further important point is that when the organisms begin to increase by an appreciable amount, an increase in surface tension is also apparent. When acid fermentation reaches the coagulation stage the surface tension of such milk approaches the surface tension value of normal milk. This increase in surface tension takes place also in a smaller degree on the inoculation of not sterilized rancid milk with *Streptococcus lactis.* The experimental data obtained suggest that the increase in surface tension is due to the utilization of free fatty acids by *Streptococcus lactis* in the process of growth.

M10. A Study of Some Factors Affecting the Viscosity of Market Cream.

F. M. Skelton and H. B. Ellenberger, Vermont Agricultural Experiment Station.

A study of the factors affecting the body of market cream, conducted at this station during the past two years, has shown that cream viscosity can be increased or decreased by simple heat treatments with no microscopic evidence of changes in fat globule clumping. This fact tends to minimize the importance hitherto placed upon fat globule clumping as a major factor influencing cream viscosity.

In an attempt to explain the decrease in viscosity after pasteurization and the increase following a subsequent heat treatment, the following procedure was used:

Samples of 30 per cent raw, pasteurized and heat treated cream from the same lot were centrifuged in an International clinical centrifuge at 1800 r.p.m.'s for three hours at a temperature of 40° F. ± 5°, using 15 ml. separatory tubes. The amount of skimmilk was read directly in ml. from the graduated separatory tube. The skimmilk was then drawn off and analyzed for total solids, fat, and solids-not-fat by the Mojonnier method.

In all cases the greatest amount of skimmilk was obtained from the pasteurized cream, usually from 2 to 3 ml. more than from either the raw or heat treated cream. There was little difference in the amount obtained from the raw and heat treated cream, the latter tending to be a little lower than the former. This last relationship, however, varied somewhat from week to week. The differences in the amount of skimmilk obtained may justifiably be interpreted to mean that pasteurization decreases and heat treatment increases the amount of bound water in cream. This is in agreement with work on bound water, reported by Dahle and Pyenson.

Analyses in most cases showed both the percentage and amount of S.N.F. to be the lowest in the skimmilk from heat treated cream and significantly higher in the skimmilk from the pasteurized and raw cream, the latter being slightly the lower of the two. These results, along with the difference in the extent of packing of the fat globules as indicated by the difference in amount of skimmilk obtained tends to indicate that pasteurization reduces, and heat treatment increases the adsorption of plasma colloids on the fat globule membrane.

M11. A Comparative Evaluation of an Ice Cream Supply as It Reaches the Consumer. L. K. Crowe and P. A. Downs, University of Nebraska.

A study has been made of factory filled and fountain dipped pint packages of vanilla ice cream as available to the consumer in a municipality of 80,000 population. Thirty-seven pint samples from nineteen manufacturing sources are included.

The study includes a comparison of these samples on the basis of cost, net weight, calculated overrun, total solids, butterfat, protein, carbohydrate (calculated), calories (calculated), pH, total bacteria count, *B. coli*, flavor score and body score. The samples are compared as purchased and after adjustment to a standard price basis.

M12. Measuring the Quality of Ice Cream. W. H. MARTIN, F. E. NELSON, AND W. J. CAULFIELD, Kansas Agricultural Experiment Station.

During the summer of 1938 pint or quart samples of vanilla ice cream were collected from 318 manufacturers by the dairy division inspectors of the Kansas State Board of Agriculture. These samples represented most of the ice cream manufactured in the state. The standard plate count, the determination of *Escherichia-Aerobacter* organisms using brilliant green lactose peptone bile broth, Scharer's rapid phosphatase test, the Minnesota butterfat test and the determination of the weight per gallon were made on each sample. Each sample was scored for flavor, texture, color and package.

Plate counts of 100,000 or less per ml. were obtained on 56.2 per cent of the samples, and 17.3 per cent of the samples had counts below 10,000 per ml. Little difference was noted between the counts on samples from wholesale manufacturers and from counter freezer operators. Only 13.7 per cent of the ice creams contained no *Escherichia-Aerobacter* organisms in 1 ml. quantities, and 21.5 per cent contained these organisms in 0.001 ml. quantities. Some correlation was noted between numbers of *Escherichia-Aerobacter* organisms and total plate counts.

The phosphatase test was of little value unless controls were run on all "positive" tests to determine whether the result was due to the presence of the phosphatase enzyme or to materials added to the ice cream. Of the 143 samples giving "positive" phosphatase tests only 17 were true positives, the other 126 being positive because of interfering substances present. The 17 samples which gave true positive phosphatase tests showed a marked tendency to be high in total plate count and in number of *Escherichia-Aerobacter* organisms and low in score.

The butterfat tests indicated that most manufacturers were complying with the legal standard for ice cream. The range of weight in pounds per gallon indicated that many samples of ice cream were high in overrun, and this adversely affected the quality of the product.

Correlations between some of the various tests were frequently observed, but no one test could be relied upon to give a satisfactory evaluation of the quality of a given ice cream sample.

M13. Consumer Preference for Individual Chocolate Varieties. P. S. LUCAS AND I. A. GOULD, Michigan State College.

No literature exists on the distinctive qualities the use of unblended varieties of chocolate may add to ice cream although characteristics of cocoa beans, grown in different sections of country along the equator, have been noted in the several texts on the subject. In this study chocolate liquor was milled without blending or flavor addition from the following beans available on the New York market: F. F. Accra (Africa), Superior Bahia (Brazil), strong type Sanchez (West Indies), Arriba (Ecuador), LaGuaire Caracas (Venezuela), fine type Trinidad (West Indies), and Puerto Cabello (Venezuela). These were analyzed for fat and ash content, using several methods for determination of the former.

Each was homogenized into separate mixes at the rate of two, two and a half, and three pounds per five gallons of mix. Samples were saved for scoring by experienced judges and by the public. Two hundred ninety-eight consumers scored each of the seven samples. Trinidad was preferred by most consumers; Puerto Cabello rated second; Bahia, third; Sanchez, fourth; Accra, fifth; Caracas, sixth; and Arriba, least. The weighted average scores were respectively, 4.4865, 4.4295, 4.1879, 4.1241, 3.8288, 3.7181, and 3.1879.

Determinations of pH were nearly identical as were fat content. Variation in color and flavor were quite noticeable and to a greater degree than shown by the composite judgment of consumer scores.

M14. The Use of the Petrographic Microscope in Studying the Different Types of Lactose Crystals as They Occur in Sandy Ice Cream.*

W. S. Arbuckle, C. W. Decker and W. H. E. Reid, Missouri Agricultural Experiment Station.

A number of different types of lactose crystals varying in shape and size have been found in sandy ice cream. It seems that the optical characteristics of these crystals however have not been definitely determined. The purpose of this paper is to present the results of a preliminary investigation concerning the optical properties of the different types of "sand" crystals as determined by the use of the petrographic microscope.

"Sand" crystals were isolated from ice cream containing 15.5 per cent serum solids which had been heat shocked to promote "sand" formation. These crystals were separated from the ice cream by centrifugal force and then washed in a saturated solution of lactose and then in acetone. The optical properties including refractive indices, optical character, optical angle, interference color, cleavage and dispersion were determined on the crystals.

The alpha hydrate form of lactose was positively identified by the optical date obtained. The bata anhydrate form of lactose has been tentatively identified although a survey of the literature failed to reveal any optical data on this form of lactose.

Chemical, as well as optical observations, indicate that very little if any sucrose is present in "sand" crystals.

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* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 604.

M15. The Use of Motion Pictures in Studying the Influence of Several Factors Upon the Stability of Ice Creams and Sherbets.* W. H. E. Reid, W. S. Arbuckle and C. W. Decker, Missouri Agricultural Experiment Station.

Studies have been made in regard to the use of ordinary colors and chromatic films taken with motion picture equipment showing the relation of variable mix compositions, effects of several factors in the manufacturing procedure and subsequent treatment to which ice creams may be subjected.

This investigation has been limited to the application of motion pictures in studying the effect of several factors upon the stability of ice cream and sherbets. The ice creams and sherbets studied include those of common flavors, also fruit and nut ice creams.

The use of motion pictures permits acquisition of more complete detailed data than is obtainable when the usual procedure is followed. Availability of data so recorded emphasizes certain factors which may not otherwise be observed.

The report of this investigation will be illustrated by the use of motion pictures showing the effectiveness of this procedure.

M16. A Microscopic Technique for Studying Fat Globules in Dairy Products and Other Oil In Water Emulsions. W. C. Cole and F. R. Smith, University of California.

In certain branches of the dairy industry it is important to know the size of the dispersed fat globules. Although several methods have been proposed to aid in such observations there is usually some difficulty in clearly differentiating the fat globules from the continuous phase. The basis of the method proposed here is that it clearly differentiates the fat globules from the background in this type of emulsion by staining the fat globules red and the continuous phase blue.

Several stains were tried to see if they might be of value in distinguishing the fat globules from the rest of the emulsion. Of those used Nile blue sulfate was the most satisfactory. It has been established that commercial Nile blue consists of a blue and a red form of the dye. The blue portion is soluble in water while the red form is preferentially soluble in fat. It is also known that the red form of the dye is encouraged by an alkaline medium.

In this study it was found desirable to dissolve the dye in a solution made slightly alkaline by the addition of sodium bicarbonate. Without taking this precaution the proportion of the red form of the dye may be insufficient to properly stain the fat. The method used is briefly described as follows: (1) Dilute the material to be examined in distilled water (usually 1 to 100). (2) Place approximately one-fiftieth of a cc. (two loop fulls) of this diluted material on a clean microscopic slide. (3) Add about one-hundredth of a cc. (1 loop full) of an alkaline aqueous solution of 1 per cent Nile blue sulfate and mix well with the sample on the slide. (4) Place a cover slip over the material and examine under the microscope.

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* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 603.

This method has been successfully used for examining milk, cream, chocolate milk, ice cream mix and evaporated milk as well as for observing fat globules in certain artificial emulsions such as mayonnaise.

M17. The Effect of the New Standard Agar on the Plate Counts of Dairy Products. F. E. Nelson, Kansas State College.

The new medium for the standard plate count of dairy products which was adopted by the Committee on Standard Methods for the Examination of Dairy Products of the American Public Health Association as the official medium after July 1, 1939, was compared with the present standard agar and with tryptone-glucose-skimmilk agar to determine its influence on the results of the standard plate count. Counts were made on 38 samples of raw milk, 27 samples of pasteurized milk, 7 samples of pasteurized cream and 32 samples of ice cream, using each of the three media at both 32° and 37° C. incubation temperatures.

The logarithmically determined average ratio of the counts on the new agar to the counts on the present agar, using the 37° C. incubation temperature, was 1.360 for the raw milk samples, 1.767 for the samples of pasteurized milk and cream and 1.350 on the ice cream samples. The logarithmic average of the ratios of the counts on the new agar to the counts on tryptone-glucose-skimmilk agar was almost exactly 1.000 in each series, being 1.034 for raw milk, 1.025 for pasteurized milk and cream and 0.992 for ice cream. Significant decreases in count resulted from the use of the improved agars in a few cases, but at least small increases in count usually were observed under these conditions. The logarithmic average increase in count due to the use of 32° C. as the incubation temperature was appreciably less when the new agar was used than when the plates were poured with the present agar if pasteurized samples were plated, but no such difference occurred when unpasteurized samples were plated.

The colonies which developed on the new standard milk agar usually were considerably larger and more easily counted than those developing on the present standard milk agar, being about the same as those which develop on tryptone-glucose-skimmilk agar. Pin-point colonies were almost never encountered when using either of the improved agars, while they occurred rather commonly on the present standard milk agar.

Higher standard plate counts may be expected in most cases as a result of the substitution of the new standard milk agar for the one now in use, the increase being about equal to that which would be obtained by using tryptone-glucose-skimmilk agar. In some cases the increase in count undoubtedly will be in excess of 300 per cent. Studies of typical organisms which fail to develop countable colonies on the present agar but are able to grow satisfactorily on the new medium are now in progress.

M18. The Development on New Standard Milk Agar of Certain Organisms of Importance to the Dairy Industry. V. D. Foltz and F. E. Nelson, Kansas Agricultural Experiment Station.

Determinations were made of the comparative growth on the new standard milk agar and old standard milk agar of a series of pathogenic and non-pathogenic gram positive cocci and bacilli of possible importance in the dairy industry. Serial dilutions of saline suspensions of the organisms grown on sugar-free beef infusion agar slants were plated on the two agars. Incubation was at 37° C. for 48 hours.

Of 2 Lancefield group A streptococci (*S. scarlatinae*) both developed colonies easily distinguishable by aid of the Quebec counter on the new medium. No colony visible with a magnification of 30X developed on the old medium.

With 53 Lancefield B. (*S. mastiditis*) cultures all developed easily countable colonies on the new medium. Using the old medium 5 cultures developed colonies which could be recognized when using the Quebec colony counter. Twenty cultures gave colonies which could be recognized as such with a 10X magnification, 10 cultures necessitated the use of 30X magnification whereas 18 cultures apparently did not develop at all.

Seven Lancefield C. (animal pyogenes)' streptococci grew on the new medium forming colonies which were easily countable using the Quebec counter. No growth was observed in the old medium.

On the new medium with one culture each of *S. durans, S. fecalis, S. lactis* and *S. zymogenes* easily countable colonies developed whereas no colonies countable with the Quebec colony counter developed on the old medium. Four cultures of *L. bulgaricus* failed to develop colonies on the old medium and only one of these cultures developed countable colonies on the new medium. With two cultures of *L. acidophilus,* one failed to develop detectable colonies in the old medium whereas the other culture developed small almost indistinguishable colonies. This was in contrast to very easily countable colony development in the new medium. One culture of *S. thermophilus* failed to give detectable colonies in either medium. With 5 cultures of *S. liquefaciens* the new medium developed detectable colonies in four instances. On the old medium one of these cultures did not form detectable colonies, while the other four cultures developed minute pin-point colonies which ordinarily would not be counted. Three cultures of *L. casei* failed to develop in the old medium in comparison to the development of difficultly countable colonies in two of the three strains in the new medium.

Many of the bacteria which are important in dairy products fail to develop countable colonies on the old standard milk agar, but grow well enough on the new standard milk agar to form colonies which are quite easily countable. Some of those organisms which fail to develop satisfactorily on the new medium belong to the same species as do some of those which do form countable colonies, indicating some variation among strains.

M19. Regulatory Legislation and Its Relationship to Research in Manufactured Dairy Products.

CHARLES S. TRIMBLE, U. S. Bureau of Dairy Industry.

The question of the proper relationship between existing regulatory law pertaining particularly to manufactured dairy products and modern educational research is one which, because of the complex nature of modern manufacturing methods as at present used, requires or should require careful study and consideration on the part of those engaged in dairy products research. The line of demarcation between legal and illegal manufacturing processes in the light of existing regulatory law is often difficult to define; therefore it becomes more and more important for research agencies to consider the regulatory angle very carefully before undertaking any extensive research. The problems to be considered are twofold in that both research and regulatory agencies have their own separate problems to face. Regulatory laws which might be termed basic and which have been on the statute books for a long time when compared to their relationship to earlier and present day research show much room for a changed attitude on the part of all concerned. Basic research should not be restricted simply because of possible long existing legal obstacles to the use of new facts for what may be illegal today may conceivably be declared legal tomorrow. A comparison of strictly commercial research and patented processes as contrasted with basic educational research and examples of both kinds of research are described. A brief history of the background and reasons for such basic regulatory laws as oleomargarine law of 1886; filled cheese law of 1896; process or renovated butter and adulterated butter laws of 1902 and the filled milk law of 1923 are given with their present day application in so far as today's research are concerned. There should not be any conflict between basic research for new facts and existing regulatory law, neither should the research investigator advocate the manufacture of any product which regulatory agencies hold to be in direct violation of the letter or spirit of existing laws or regulations. The possible need of revision or amendment of some existing laws is briefly discussed if real progress in research is to be attained. No specific recommendations or discussion of relative merits of present day practices is attempted but the whole subject of relationship treated generally as being worthy of possibly more careful attention in the present than has been given to the question in the past.

M20. Studies with the Rollerless Churn. W. B. Combs and S. T. Coulter, University of Minnesota.

The rollerless churns on the market are finding favor in American creameries. The work here reported presents results secured on observing the churning efficiency of these churns as compared with a standard single roll churn. Comments are offered on the body and texture and the uniformity of the composition of butter churned and worked in the rollerless churn.

M21. The Effect of the Ration on Butter Fat Constants. A. H. Kuhlman and W. D. Gallup, Oklahoma A. & M. College.

The results obtained with grade Jersey cows used in an investigation of the vitamin A requirements of dairy cattle offered an opportunity to study several factors which influence the physical and chemical properties of butter fat. These cows received a basal ration consisting of several levels of intake of prairie hay, molasses beet pulp, and cottonseed meal with bone meal as a mineral supplement. Daily allowances of 4, 8, and 11 pounds of prairie hay represent the levels of hay intake in three of the rations used with molasses beet pulp and cottonseed meal. Another ration consisted entirely of prairie hay and cottonseed meal fed *ad libitum.*

Separate churnings were made from the cream of the combined morning and evening milkings of individual cows at 30-day intervals during the entire lactation period for eight cows. For the butter fat obtained from each of these churnings, fat constants were determined for the melting point, melting time, hardness, and iodine value.

The results reveal individual variability with respect to the properties of the fat produced by cows on the same feed, and in the same period of lactation. Likewise, there were wide differences between samples from the same individual. The combination of cottonseed meal and prairie hay produced a hard butter fat with a high melting point, and slightly lowered iodine value. Butter fat produced in the early periods of lactation was usually softer, and had a lower melting point, and higher iodine value than that produced in the later periods. Molasses beet pulp when fed at the highest level was effective in decreasing butter fat hardness.

M22. Some Observations on the Iodine, Reichert-Meissl and Polenske Values of Milk Fat Produced During the Winter in Three Sections of Oregon. R. E. Stout and G. H. Wilster, Oregon State College.

A comparison was made between the iodine, Reichert-Meissl and Polenske values of milk fat produced under winter feeding conditions in three agricultural areas of Oregon: the coast section where native grass, clover, vetch and oat hays, silage, root crops and some mixed grains are fed to cows during the winter; the Willamette Valley section where clover, vetch and oat hays, some alfalfa hay, root crops, silage and some grain are fed; and the eastern and southern Oregon section where alfalfa hay forms the major portion of the winter ration. Samples of butter made during January, 1939, were obtained from 15 representative creameries for analysis.

In general, the butter obtained from the coast and Willamette Valley sections had a medium-firm body and a close texture, while that manufactured in the eastern and southern Oregon section had a firm body and showed a crumbly and sticky texture.

Very little difference was found to exist in the unsaturated fatty components of the milk fat produced in the three above sections. The iodine values of the fat from all sections ranged from 29.75 to 32.5 and averaged 31. The iodine values of the fat produced in the Willamette Valley ranged from 29.75 to 31.43 and averaged 30.55, those of fat from the coast ranged from 31.38 to 32.5 and averaged 31.89, while those of fat from Eastern and Southern Oregon ranged from 29.95 to 31.70 and averaged 30.74.

Milk fat from the coast had Reichert-Meissl values that ranged from 29.25 to 31.5 and averaged 30.65, and Polenske values that ranged from 2.09 to 2.34 and averaged 2.23. Fat from the Willamette Valley had Reichert-Meissl values that ranged from 30.78 to 31.45 and averaged 31.10, and Polenske values from 2.01 to 2.30 with an average of 2.18. Fat from Eastern and Southern Oregon had Reichert-Meissl values that ranged from 26.03 to 27.89 and averaged 27.02, and Polenske values from 1.71 to 1.85 with an average of 1.78.

M23. A Preliminary Report on Investigations to Overcome Crumbly and Sticky Texture in Butter Made During the Winter in the Irrigated Sections of Eastern and Southern Oregon.

G. H. WILSTER, R. W. STEIN AND R. E. STOUT, Oregon State College.

The defect, crumbly and sticky texture of butter, affects the butter made in 34 creameries in the irrigated alfalfa producing sections of Eastern and Southern Oregon. A large percentage of the butter made in these creameries during the winter is shipped in 68 pound cubes to various markets where it is sold in competition with butter that does not possess the undesirable texture defect. This results in an unfavorable market acceptance, and the butter, when it shows the defect to a noticeable degree, is sold at a lower price.

The following are some of the results that have been obtained to date:

1. The use of cold wash water made the butter granules very hard. A much longer time was required for completing the working process. There was an increased danger of stickiness by this method, but in some instances the crumbly tendency was reduced. Under the condition of the investigation, this was not a reliable corrective method by itself.

2. When the cream was held overnight at a low temperature, such as below 48° F., after pasteurization and cooling, even though it was churned at a relatively high temperature such as from 58° to 60° F., the hardness of the butter was increased. The crumbly character became more pronounced. Higher holding temperatures resulted in softer-bodied butter, but they did not prevent stickiness or crumbliness.

3. The length of time of holding the cream after pasteurization and cooling until it was churned had an effect on the texture of the butter. Some improvement in texture was noticed when the cream was churned immediately after pasteurization and cooling or when not held longer than periods up to two hours after pasteurization and cooling. When the pasteurized, cooled cream was held from 14 to 18 hours, then heated to 120° F., cooled rapidly in a coil vat to a suitable churning temperature, and churned immediately, it resulted in butter that was relatively free from stickiness, but it showed the firm, brittle characteristic after it had been cooled to a temperature of 45° F. in a refrigerator.

4. Slow working of the butter in some cases improved the texture of the butter. This method was very slow and required up to two hours for a complete working. The butter received a much longer and less severe working, which seemed to be beneficial.

5. The rate of cooling of the cream after pasteurization had a marked effect on the butter in the churn. Rapid cooling to a low temperature (below 48° F.) caused the granules and the butter to be very firm. Rapid cooling to 70° F. and then slowly to 48° F. (about 45 minutes) caused softer granules and softer butter. Less crumbliness was noticed in the butter made from the slow-cooled cream.

6. Flash pasteurization seemed to have little effect in improving the butter texture.

7. Butter was taken from the churn and held for several days at a temperature of 40° F., 50° F. and 70° F. respectively. After holding at these temperatures, the butter was tempered over a period of two days to 50° F. and examined. The butter that had been held at 40° and 50° F. showed stickiness but little crumbliness, while the butter that had been held at 70° F. was reasonably free from stickiness but showed pronounced crumbliness.

M24. Copper in Butter. B. E. HORRALL AND W. F. EPPLE, Agricultural Experiment Station, Purdue University.

A year's study has been completed on the copper content of butter. The samples used in this study were from approximately fifty plants, manufacturing butter in Indiana and neighboring states. These samples were sent in monthly for analysis.

The copper content was determined by our modification of the Ritter method. This modification consisted of adding one-half ml. of copper-free water and eliminating the addition of milk. No incubation was necessary with this procedure. The test is as follows:

Place about 10 grams of unmelted butter in a test tube and add one-half ml. of copper-free distilled water. This mixture is heated at 90° C. for five minutes. The samples should be placed in a water bath previously heated to 90°–91° C. and this temperature maintained throughout the holding period to kill or inactivate any peroxidase present.

Solutions (1) 0.2 gm. para-amino-dimethyl-anilin sulfate is added to 5 cc. of copper-free water (glass distilled) and heated to 90° C. in the presence of bone black (1 gm.) and then filtered. (Made fresh every day.) (2) 1% of alpha naphthol in alcohol. (3) H_2O_2 1% solution in glass distilled water.

Add to the melted butter one drop of each of the above solutions, shaking after adding the first two. Then add one drop of the H_2O_2 and shake again. Watch the separation of the curd and time the color begins to appear. In the very positive copper samples, the purple color appears almost at once. "Positive" in about five minutes and "trace" in 10–15 minutes. We have found that if the color does not develop in 15 minutes, it is normal copper content and called "negative."

This qualitative method was compared with Williams quantitative method using a neutral wedge photometer for the measurement of the developed color.

1. There was an average of 21.3 per cent of the samples showing positive copper, while 39.1 per cent showed slight copper and 39.6 per cent negative copper.
2. A keeping quality test (70° F. for seven days) was made on all of the butter and the results indicated that the positive samples had inferior keeping quality.
3. Most of the butter with positive copper was of poorer quality when received.
4. The pH of the butter interfered somewhat with the test. With most of the high pH samples (7.1–7.5) the test was unreliable. The curd was generally suspended and remained in the fat, with the result that such butter gave a negative test to the modified Ritter test even though the copper content was high.
5. The comparison of the modified Ritter test with the quantitative test (Williams) indicated that the negative copper samples ranged from 0.0 to 0.58 parts per million and averaged 0.229; the slight copper samples ranged from 0.19 to 0.54 and averaged 0.291 parts per million, while the positive copper samples ranged from 0.32 to 2.33 and averaged 0.583 parts per million.

M25. Acid Values and Acid Ratios as Related to the Keeping Quality of Salted Butter. H. A. Bendixen, Washington Agricultural Experiment Station.

The acid values were determined for 28 samples of butter made from sweet cream and 51 samples made from neutralized cream by titrating a boiling mixture of 10 grams of butter and 50 ml. of neutral ethyl alcohol with N/50 Na OH using phenolphthalein as an indicator. In a similar manner the acid values were also determined for the butterfat obtained from the above samples of butter by centrifugation of the butter melted at 45° C. The acid ratios were determined by dividing the butter fat acidity by the butter acidity and multiplying by 100.

High acid values of butter and butter fat before and after storage showed a slight tendency toward reduced keeping quality of the butter at 0–5° C. High increases in the acid values after storage at 21° C. and 0–5° C. did show a fairly close correlation with reduced keeping quality of sweet cream and neutralized cream butter, at 0–5° C, but especially with sweet cream butter.

An increase in the acid ratio during one week at 21° C. and during one month at 0–5° C. seemed to be very closely related to poor keeping quality of the butter at 0–5° C., especially in the case of sweet cream butter.

M26. The Influence of Various Factors on the Reliability of the Room Temperature Holding Test for Butter Quality. D. H. JACOBSEN, S. Dakota State College.

In an effort to determine the cause of the lack of agreement between the results of the holding test at room temperature and the keeping quality at lower temperatures, a series of comparisons were made on both salted and unsalted butter. The room temperature holding tests were made by holding five ounce samples in a thermostatically controlled box at 21 ± 1° C. Similar samples of the corresponding butter were held at 4.4° C. in a laboratory refrigerator and at approximately 0° C. in a butter holding room.

The samples were examined for flavor deterioration at intervals depending on the temperature of holding. The factors considered were the salt content, the yeast and mold count and the total, lipolytic and proteolytic bacterial counts. The microbiological examinations were made both before and after holding at the various temperatures.

Seventy-eight lots of butter from South Dakota creameries have been studied. These tubs represented churnings made for the educational scoring contests held at the College over the last three years. In addition to these, thirty-six laboratory churnings have been made using cream inoculated with cultures of known lipolytic or proteolytic activity.

The results indicate that, in general, the activity of the organisms was effected to a great degree by the salt content of the butter. The butter containing salt above the one per cent level showed very little deterioration which could be correlated with the activity of microorganisms. Also the proportion of the organisms which were either lipolytic or proteolytic was greater in the butter held at the lower temperatures. The lack of agreement between the room temperature holding test and keeping quality at lower temperatures, therefore, appeared to be due to the differences in the active bacterial flora at these temperatures. This was particularly true with butter of salt content below one per cent and with unsalted butter.

M27. Laboratory Equipment for High Temperature Pasteurization Studies. S. T. Coulter and W. B. Combs, University of Minnesota.

Commercial equipment for experimental work in high temperature pasteurization is prohibitive in original and operation cost for most Experiment Stations. A pasteurizer, very moderate in cost and sufficiently small so that lots of cream such as would be available in feeding trials with individual cows can be readily handled, has been constructed and operated. The basic principle of operation is the same as that used in the high temperature pasteurizer made by the C. E. Rogers Co. of Detroit, Michigan. The equipment consists essentially of a glass tube connected at one end by means of rubber tubing with sources of cream and steam both under 50 to 60 pounds pressure, and at the other end by the same means to a flask upon which a partial vacuum can be drawn.

The glass tube is 3 feet long and is made of standard 12 mm. O. D. Pyrex tubing. The cream and steam enter the tube through the arms of a Y. Tubes drawn to a 3 mm. orifice are fitted into the arms of the Y in order to cause the cream and steam to meet as sprays. The rate of passage of the heated cream from the tube is regulated by a stopcock placed near the end opposite that at which the steam and cream enter. A thermometer is inserted into the tube by means of a ground glass connection and is held in place by spring clips. Leaving the tube the cream enters a 6 liter Erlenmeyer flask through an opening about half way up the side of the flask. The vapors are drawn off and passed through a surface condenser and a partial vacuum maintained by means of a water aspirator. All glass blowing was done by E. F. Greinke, of the Scientific Apparatus Shop, University of Minnesota.

In operation steam under pressure is admitted to the tube and after flow is established the outflow restricted by the stopcock until a temperature of at least 129° C. (265° F.) is reached. Cream under pressure is then admitted to the tube at such a rate as to maintain the temperature at 129° C. (265° F.) or above.

Numerous lots of cream so pasteurized gave zero bacterial counts using the standard plate method, and were practically free from cooked flavor.

M28. Correlation Between Grades on Scores and Grades on Criticisms in the Judging of Dairy Products. G. M. Trout, Michigan State College.

Studies were made on the grades obtained of 69 contestants in the judging of seven samples each of butter, cheese, milk, and ice cream in the 1938 National Dairy Products Judging Contest. The grades represent 483 sample-judgments per product. Grouping of contestants according to their abilities to score a product indicated little difference between the good and poor judges in their abilities to criticize the product; whereas grouping them according to their abilities to criticize the samples showed that these who had ability to criticize samples also had ability to score the product. Correlation coefficients between grades on "scores" and grades on "criticisms" for 69 individuals were .31, .82, .32, and .45 for butter, cheese, milk and ice cream respectively. Those for the team grades were .87, .67, .26, and .46 for the same respective products. Charts showing lines of regression between team grades on scores and grades on criticisms are included.

M29. Standards for Rating Cheddar Cheese.

J. C. Marquardt, New York Agricultural Experiment Station, Geneva, N. Y.

Recent studies at the New York Agr. Exp. Station have revealed that the salt percentage in cheddar cheese influences its flavor, texture, and fitness for long curing.

It is proposed, therefore, to present a score card that will allow 5 points for salt. Regardless of whether the cheese is rated on flavor with 50 or 45 points, and on texture with a corresponding 25 or 30 points, the 5 points allowed for salt should reduce the perfect finish from 15 to 10.

In view of established facts the following scale is proposed for rating cheese on the salt basis.

CHEESE SCORE CARD

Flavor	50
Texture and body	25
Color	10
Finish	10
Salt	5

Salt in cheese—Perfect Score, 5

	Points
1.50–1.70 per cent	5.0
1.30–1.49 or 1.71–2.00 per cent	4.9
1.10–1.29 or 2.01–2.20 per cent	4.5
0.90–1.09 or 2.21–2.40 per cent	3.5
Below 0.90 or above 2.40 per cent	0.0

C. F. (cheese flavor rating)—

Allowing 5 points for a perfect rating on salt is a minor but important change in rating cheddar cheese. Its main advantage is as a guidance to a proper salt content in the cheese. Obviously when cheeses are more generally analyzed for salt either by control or commercial laboratories, the makers will become aware of the fact that quality and a proper salt content are closely related.

The item of cheese flavor is important and should be included on score cards. For example, cheeses with comparable scores may vary in cheese flavor. Lacks cheese flavor, mild cheese flavor, good cheese flavor or strong flavor are suitable designating terms which can be empirically expressed in corresponding values of 50, 60, 75 or 95. Using these numbers with a cheese score may be confusing. However, two possibilities for recording C. F. (cheese flavor) are presented.

M30. Observations on the Growth Responses of *S. Lactis* in Mastitis Milk.

C. C. PROUTY, Washington Agricultural Experiment Station.

The experiments reported are confined largely to studies made on the milk drawn from the normal and diseased udder quarters of three cows.

Milk with an initial pH value greater than 6.9 usually failed to support the growth of *S. lactis* in an active manner thus resulting in delayed acid production. Numerous samples with pH values greater than 7.0 showed no appreciable growth of *S. lactis* whereas normal samples from the other udder quarters showed normal acid development. Adjusting the pH value of mastitis milk to that of normal milk resulted in only partially overcoming delayed acid development. The addition of mastitis milk to normal milk in as little as 10 per cent concentrations had a retarding effect upon *S. lactis* development. Pasteurizing some samples at 150° F. and 155° F. for 30 minutes partially overcome the retarding influence on the growth of *S. lactis* but with most of the samples little or no effect was evident.

M31. Potentiometric Studies with Resazurin and Methylene Blue in Milk.

C. K. JOHNS AND R. K. HOWSON, Department of Agriculture, Ottawa, Canada.

Resazurin reduces to the pink stage in approximately three-fourths of the time required for methylene blue to decolorize. Potentiometric studies indicate that this shortening of reduction time is attributable to the changes in shape of the time-potential curve when resazurin is present. The resazurin-milk curve reaches the zone of reduction earlier than does methylene blue. This is followed by a flattening of the curve due to the greater poising action of this dye.

Hourly inversion of tubes during incubation generally shortens the reduction time considerably. The incorporation of oxygen by this practice has little or no effect upon the time-potential curve during the early stages, and only a transient effect later.

Resazurin is more useful than methylene blue in detecting milks containing large numbers of weakly reducing organisms.

The lengthening of reduction time when the concentration of methylene blue is increased from 1 : 700,000 to 1 : 300,000 appears to be attributable to a downward shift in the Eh zone at which the dye decolorizes.

M32. Lactic Acid Streptococci of Cheese Starters.

BLYTHE A. EAGLES AND OLGA OKULITCH, The University of British Columbia, Vancouver, Canada.

A study of streptococci isolated from cheese starters has been made, employing methods used by different workers in this field for the classification of the microorganisms.

The ability of the organisms to grow in methylene blue milk, in 4 per cent sodium chloride broth and in nutrient broth of pH 9.2 after the manner of Sherman; the nature of their growth in glucose broth as described by Davis; their vitality as measured by the technique of Whitehead, and their ability to ferment carbohydrates as measured quantitatively according to the method of Orla-Jensen have been determined.

The relationship of these procedures insofar as classification of the Lactic Acid Streptococci is concerned is considered.

M33. Application of the Smear Culture Technique to the Examination of Cheddar Cheese.

C. B. Lane and B. W. Hammer, Iowa State College.

The plating of cheddar cheese on various media has been useful in showing the general changes in numbers of bacteria during manufacture and ripening but has not been particularly helpful in studying some of the bacteriological problems involved in cheese ripening. The advantages of the smear culture technique in the examination of butter and ice cream suggested its application to cheese.

In carrying out the technique uninoculated plates were poured and allowed to solidify. The weighed sample of cheese was ground with 2 per cent aqueous sodium citrate and dilutions made in sterile water. One-tenth ml. portions of each dilution were smeared on the plates with sterile bent glass rods. After inoculating the plates at 21° C. for about 1 week they were examined for the numbers and general types of colonies present.

The technique was compared with the usual plating procedure. Beef infusion agar was employed with both methods since preliminary trials had indicated it was more generally satisfactory than agars prepared from tomato juice, cheese infusion or milk.

The general results of the comparison are as follows: 1. Higher total counts were generally obtained with poured plates. 2. Colonies were larger and developed more rapidly on smeared plates. 3. More of the rapid acid producing organisms, particularly *S. lactis,* were found on poured plates, whereas more of the chromogenic cocci and protein digesting types were regularly present on smeared plates.

The smear culture technique appears to have advantages over the plating procedure in studying the bacteria other than the homofermentative lactic acid organisms in cheese.

M34. Relationship of Fat Hydrolysis to Flavor Development in Cheddar Cheese.

C. B. Lane and B. W. Hammer, Iowa State College.

The relatively large acid numbers obtained on fat from aged cheddar cheese, and also the higher acid numbers accompanying the more rapid flavor development in raw milk cheese than in pasteurized milk cheese, suggest that changes in the fat may be important in the ripening process.

In order to study hydrolysis of the fat, cheeses were prepared from normal milk (raw or pasteurized) and also from milk which had been separated, the cream homogenized (raw or pasteurized) and then recombined with the skim milk (raw or pasteurized).

A rancid flavor regularly developed very early in cheese from homogenized raw cream plus raw skim milk and, to a lesser degree, in cheese from homogenized raw cream plus pasteurized skim milk. As ripening progressed, the rancid flavor rapidly diminished; after 4 to 6 weeks, it has completely disappeared. Following disappearance of the rancid flavor, a flavor characteristic of aged cheddar cheese developed; after 8 weeks, considerable flavor was present. Cheese from homogenized raw cream (plus raw skim milk) regularly developed more flavor than cheese from homogenized pasteurized cream (plus pasteurized skim milk) or from normal pasteurized milk; in most instances it developed more flavor than cheese from normal raw milk. The results suggest the importance of milk lipase in the ripening process.

Since a rancid flavor rapidly developed and disappeared in the cheese from homogenized raw cream (plus skim milk) it is evident that factors were present which resulted in the destruction of the fatty acid or acids responsible for the rancid flavor. Presumably, these factors are also operative in cheese made from normal milk and because of the relatively slow production of the acids a rancid flavor does not develop.

M35. Some Factors Affecting the Moisture Content of Swiss Cheese and Their Effects upon Quality and Yield. GEORGE P. SANDERS AND ROBERT R. FARRAR, U. S. Bureau of Dairy Industry.

Data on nearly 200 laboratory Swiss cheeses, each weighing about 60 pounds and cured 2½ to 3 months, indicate that the inclusion of more than 39.7 per cent moisture in green cheese usually results in serious detriment to the quality of the cheese, particularly with respect to eye formation. The conclusion that high moisture is detrimental to quality is substantiated by results of analyses of nearly 400 factory cheeses in 3 years' study in producing areas in Wisconsin and Ohio.

Conditions which were found to increase the percentage of moisture in laboratory cheese are: low-fat milk, soft-curd milk, addition of sodium citrate or of water to milk, slow pH development caused by a decrease in activity of streptococcus starter or by "slow-working" milk, decrease in quantity of rennet, low setting temperature, early cutting, fine harping, rapid heating, a combination of low cooking temperature and short stirring-out time, and a general shortening of time of each stage of the making process.

Increases in percentages of moisture did not result in corresponding increases in yield. Some of the above factors which cause increases in moisture content in cheese likewise cause abnormal losses of fat and other solids in the whey; such factors include the use of soft-curd milk, "slow-working" milk, slow pH development, and fine harping. Removal of an excessively large proportion of fat from the milk produces, in addition to low yield, high moisture content and poor quality. High-moisture cheeses undergo a relatively large loss in weight during curing.

M36. Further Studies on the Gas Requirements of Three Strains of Blue Mold (*Penicillium Roqueforti Group*) Isolated From Cheese.

N. S. Golding, Washington Agricultural Experiment Station.

The paper presents further data, obtained during the last two years on the gas requirements of blue mold (Penicillia) isolated from blue veined cheese. The data are expressed by growth curves and each point represents the average measurement of the diameter of five colonies incubated for each of seven different temperatures under identical conditions of gas supply. A seven-day incubation period and the same lot of Difco malt agar were used for all growth curves. With all curves growth is selected as the Y-axis and temperature as the X-axis. Thus by interpolation from the growth curves, other curves can be made for any growing temperature, with growth as the Y-axis and concentration of any one of the gases used as the X-axis. Tables also are interpolated from the growth curves for definite temperatures of growth. In these tables the control, air, is given the value of 100. Significant deviations are at once apparent when using any known gas mixture.

The growth curves of three strains of *P. roqueforti* are given for six dilutions of air (less CO_2) with N_2.

The curves show:

Where air is diluted up to 50 per cent added N_2 by volume, no significant difference in growth is recorded.

With a gas supply of air 30 per cent and added N_2 70 per cent, a very slightly significant inhibition in growth with one culture is recorded. The other two cultures are not significantly inhibited.

A gas supply of air 20 per cent and added N_2 80 per cent, produced no inhibition in growth at 60° F., slight inhibition in growth at 70° F. and a somewhat greater inhibition at 80° F.

Up to and including air 20 per cent and added N_2 80 per cent (*i.e.*, 4 per cent O_2) inhibition did not exceed 15 per cent of the control, air.

With gas supply of air 10 per cent and added N_2 90 per cent (*i.e.*, 2 per cent O_2) growth is inhibited, however, in eight out of nine cases growth was more than 50 per cent of the control, air.

Inhibition is greater at higher temperatures of growth than at the low.

The growth curves of the three strains of *P. roqueforti*, are given for 8 dilutions of air with CO_2.

The curves show:

With small additions of CO_2 to air, up to 10 per cent and in some cases higher, there is an increase in the growth, which is variable both with temperature and strain of organism.

Up to CO_2 30 per cent and air 70 per cent inhibition in growth is not noticed with the exception of low temperatures of growth and is variable with the strains of mold.

With equal parts CO_2 and air inhibition of growth is definite at all temperatures but is very much greater at the lower temperatures than at the higher temperatures. The area enclosed below the growth curve, which represents the average growth for all temperatures is of the same order as that obtained for 90 per cent added N_2 in air (*i.e.*, 2 per cent O_2).

With CO_2 70 per cent and air 30 per cent growth is totally inhibited during one week at temperatures of 55° F. and below, while at 70° F. growth is about 50 per cent of the control, air, however, there is considerable variation between strains.

The inhibiting effect of CO_2 is much greater at low temperatures of growth than at the higher temperatures which, is directly the opposite to that obtained with N_2.

M37. The Lipolytic and Proteolytic Activities of Various Penicillia of Importance in the Ripening of Blue Cheese. C. Jensen, North Dakota Agricultural Experimental Station.

In a study of 27 strains of penicillia obtained in part from culture collections and from blue cheese isolations, considerable variations were noted in their lipolytic activities on natural fats (butterfat and cottonseed oil), and on various simple triglycerides, dispersed in Czapek's solution agar, as determined by the natural fat and the Nile blue sulfate technics.

The strains of penicillia studied also exhibited significant differences with respect to their proteolytic activities as shown by their action on skim milk contained in Czapek's solution agar.

PRODUCTION SECTION

P1. The Lactation-Promoting Properties of Proteins as Measured With Rats. J. R. Haag and L. D. Wright, Oregon Agricultural Experiment Station.

Specific information on the lactation-promoting properties of proteins is largely limited to the knowledge that a liberal protein intake is essential for a high level of lactation. The extent to which conventional biological values and growth-promoting properties are applicable to lactation is still problematical. Experiments conducted in our laboratory have thus far been largely confined to the proteins of alfalfa leaf meal, corn gluten meal, peanut meal, and to the amino acids cystine and methionine.

We have previously shown that the growth-promoting and lactation-promoting properties of alfalfa leaf meal protein are enhanced by additions of l-cystine. Of particular interest to the northwestern states is the fact that all samples of alfalfa thus far tested, regardless of sulphur fertilization history, have been improved by the addition of cystine. We have also found alfalfa crude protein to contain a relatively constant percentage of organic sulphur. The conclusion of practical interest is that while sulphur fertilization frequently increases the yield of crude protein, it apparently does not affect its biological value for growth or lactation.

Another phase of our work deals with a quantitative comparison of the influence of different levels of cystine on growth and on lactation in rats fed rations containing 9–10 per cent alfalfa crude protein. Under the conditions of these experiments we have failed to demonstrate any significant difference in the requirements for growth and for lactation.

Still another group of experiments in which the protein is furnished by peanut meal deals with the conditions governing the interchangeability of cystine and methionine. Growth and lactation are not improved by the addition of cystine but respond to methionine. Here again, we have failed to demonstrate any fundamental difference in the requirements for growth and for lactation.

The study of supplementary effects among proteins on lactation is a difficult problem. The proteins of corn gluten meal do not appear to make good the cystine deficiencies of alfalfa crude protein. Experiments are now being planned to determine the supplementary effects of various feed proteins on the lactation-promoting properties of alfalfa proteins.

P2. Recent Advances in the Endocrinology of Milk Secretion.* C. W. Turner, Missouri Agricultural Experiment Station.

One of the most intriguing problems confronting breeders of dairy cattle is the explanation of the physiological expression of the genetic differences for milk production. By what means are the cows which receive the potential ability for large milk secretion enabled to utilize and convert the large amounts of feed received into milk? Work under way at this Station leads us to believe that the variation in the secretions of the endocrine glands causes the difference. The master gland of the endocrine system is the pituitary. It plays a unique rôle in regulating the growth and secretory activity of the udder, the largest gland of the body.

The pituitary secretes a hormone called *mammogen,* which stimulates the growth of the udder slightly during the recurring estrus cycles and very greatly during the first two-thirds of pregnancy. Small amounts of mammogen may be present in the pituitary at all times but it has been shown that the ovarian hormones, estrogen and progestin, which are secreted in large amounts during pregnancy cause the pituitary to secrete large amounts of this hormone. In comparisons of beef and dairy cattle pituitaries during pregnancy, more mammogenic hormone is being found in the latter. It seems reasonable to believe that the size of the udder is controlled in part by the rate of secretion of mammogen.

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* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 602.

When the growth of the udder is completed, the secretion of milk must be initiated. A second pituitary hormone called *lactogen* starts the epithelial cells to synthesizing milk. We have discovered that the amount of this hormone increases very rapidly after calving and explains why milk production increases rapidly at this time. The pituitaries of dairy cattle have been shown to contain much more of this hormone than beef cattle.

The above hormones directly influence the udder. There are other pituitary hormones, however, which have an important indirect effect upon lactation through their action upon the absorption of food, the composition of the blood, the rate of circulation of the blood, and the activity of the udder cells. In this class comes the pituitary thyrotropic hormone which stimulates the thyroid gland to increased secretion of thyroxine. We have discovered that the thyrotropic hormone also increases markedly following parturition and is present in higher concentration in dairy cattle than in beef cattle.

Further, it has been shown that the injection of thyroxine into some dairy cattle increases the secretion of milk during the period of treatment. Does this mean that the cow's own thyroid gland is not secreting optimum amounts of thyroxine for maximum secretion?

The pituitary has been shown to secrete hormones which influence the parathyroid and the adrenal glands as well. It also influences the metabolism of protein, fat and carbohydrates and may in this way regulate the rate of milk secretion through their action upon the precursors of milk in the blood.

P3. Some Factors Affecting the Reliability of Arterio-Venous Differences in a Study of Blood Precursors of Milk.

W. E. PETERSEN AND W. L. BOYD, Minnesota Agricultural Experiment Station.

In the study of blood precursors of milk and the metabolism of the mammary gland by determining arterio-venous differences of the various blood substances, great variations in values are secured from time to time.

These variations may be due to normal changes in the blood substances, variation in the quantity of blood constituents removed by the gland or abnormal changes due to the act of taking blood samples. While the first two possibilities are undoubtedly contributing factors, the latter possibility must be given even more serious consideration.

Numerous blood volume changes were determined in arterio-venous samples in which the venous samples were taken simultaneously as well as immediately before and immediately after the arterial samples. The changes in blood volume may be either a dilution or concentration of the venous blood in relation to the arterial. As much as 5 per cent dilution and 11 per cent concentration were observed. Arterial samples taken at one minute intervals varied as much as 3 per cent. In excited animals large blood concentration changes were observed more frequently than in those not excited, although some very excited animals showed no change and some animals not visibly excited showed great changes.

Obviously when there are changes in the blood concentration the observed values should be corrected. When the arterio-venous difference of many substances are corrected for blood volume changes the corrected values often appear to be very abnormal.

Where there are no changes in blood concentration and no visible excitement of the animals, the observed variations must be explained some other way. Inasmuch as the water of the blood may go either way upon excitement, it seems probable that the act of taking blood samples may often cause pronounced almost instantaneous effects upon the blood and metabolism which may or may not be reflected in concentration changes of the blood. However, the most nearly constant arterio-venous differences in the blood precursors of milk are usually obtained when there are little or no changes in blood volume.

Variations in the arterio-venous differences can also be attributed to changes in blood flow through the gland and to endocrine and pressure effects in the letting down of milk. That there is a considerable variation in the blood flow is apparent from the large fluctuations in the amount of oxygen taken up by the gland. Data on perfusion experiments show that the failure of the gland to remove fat from the blood for an hour or so after milking is due to the endocrine effects causing the letting down of milk. Blood flow is also decreased due to this effect.

Variations in blood volume differences in arterial and venous samples are more closely associated with the excitation of the animal than are either glucose or phosphorus differences. The state of excitation of the animal is of more importance in drawing good blood samples than is the rapidity with which the samples are taken. It is suggested that only those samples showing little or no blood volume changes be used in studying milk secretion.

P4. The Respiratory Quotients of the Intact and Perfused Mammary Glands of Cows. J. C. Shaw, Minnesota Agricultural Experiment Station.

The respiratory quotients of both the intact and perfused excised mammary glands of cows were determined from arterio-venous blood samples.

The results in the case of the intact animal were difficult to evaluate because of the great variation and the necessity of correcting for blood volume changes in many cases. Correction for blood volume changes produced values that were obviously untenable. Uncorrected respiratory quotients varied from 0.56 to 2.26. Those corrected for blood volume changes varied from 0.22 to 0.986. Where no blood volume changes occurred the respiratory quotient varied from 0.93 to 1.70. The oxygen differences varied from 3.7 to 7.1 volumes per cent while the carbon dioxide differences varied from 2.2 to 8.4 volumes per cent.

For non-lactating glands the respiratory quotients were 0.20 or less in the intact gland. The oxygen consumption was much higher, reaching as much as 11.9 volumes per cent.

On the perfused excised lactating glands the respiratory quotients were much more constant, varying but little from a mean of 0.80. Although but few excised dry glands have been perfused the indications are that the respiratory quotients are about unity which is the value to be expected from the burning of carbohydrate. The respiratory quotient secured on perfused excised glands are significant in that the values for the excised dry gland are higher than the intact dry gland while for the lactating glands the values for the perfused gland are lower than the intact gland; also the values for the perfused non-lactating gland are greater than for the perfused lactating gland.

The values obtained on perfused lactating glands indicate that fats are oxidized and this lends support to the hypothesis previously advanced that milk fat is accounted for by the blood fat and that the lower fatty acids of milk fat are produced by partial oxidation of the higher fatty acids. Continued studies of the arterio-venous differences of blood fat show that sufficient fat is removed from the blood by the mammary gland to account for the milk fat.

P5. Perfusion of the Excised Mammary Gland as a Method of Studying Milk Secretion. W. E. Petersen, J. C. Shaw and M. B. Visscher, Minnesota Agricultural Experiment Station.

Perfusion, providing the excised mammary gland functions normally when perfused, offers the following advantages as contrasted to the living animal in the study of the blood precursors of milk:

1. The many factors, outside of the mammary gland, affecting blood changes are eliminated.
2. Through continued perfusion of the same blood it becomes possible to detect some of the milk precursors that are removed in minute amounts.
3. It becomes possible to study the effects of partial or complete depletion in the blood of the several milk precursors.

4. It becomes possible to study the effect upon milk secretion of substances added to the blood.
5. The rate of blood flow through the gland can be accurately determined at all times.
6. The effect of varying blood pressures can be studied.
7. It becomes possible to secure the lymph and determine its composition.

Two methods have been used in perfusion, one involving the Starling-Visscher heart-lung preparations of goats and the other an artificial heart and lung for perfusion of cow glands.

A fairly satisfactory perfusion apparatus and technique have been developed and some significant results have been obtained relative to the metabolism of the excised gland. The apparatus consists of an artificial heart, resistance for maintaining constant blood pressure, a constant water bath for maintaining blood temperatures, and an oxygenator. Both the gland and defibrinated blood are obtained from cows at the time of slaughter. The gland is suspended from the median suspensory ligament and skin. The gland seems to function normally even when as much as one hour has elapsed between the time of slaughter and the start of perfusion.

As evidence that the perfused excised gland functions normally, the following observations have been made:

Glucose is used constantly and is rapidly depleted in the blood.

Blood fat is used by the perfused gland and the same relationship between the arterio-venous difference and the time of milking as was observed in the normal cow is maintained in the perfused gland.

Nitrogenous substances are also used, and the great variations in the blood nitrogen of the normal cow which practically renders interpretations impossible are not observed in the perfused blood.

The respiratory quotient of the perfused gland in all experiments has been remarkably constant on the original blood.

With the depletion of certain blood constituents the respiratory quotient of the perfused lactating gland decreases.

P6. The Effect of Ultraviolet Radiation on Milk Production. W. E. Krauss, C. F. Monroe, C. C. Hayden, and B. H. Edgington, Ohio Agricultural Experiment Station.*

Ninety-six Holstein cows in the dairy herd at the Ohio State Reformatory (Mansfield), were classified on the basis of their agglutinin titers as determined by two agglutination tests run in successive months. The cows were classed as non-reactor, questionable, and reactor animals and were divided into two groups, each of which contained an equal number of animals showing the same reaction.

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* In cooperation with the State Department of Public Welfare and the General Electric Company, Nela Park, Cleveland, Ohio.

One group of cows served as controls while the other was irradiated with ultraviolet light generated by S–1 sunlamps which were so placed that maximum exposure occurred on the head region. In the course of a year it was calculated that the irradiated group received the equivalent of 100 hours of midsummer sunshine over and above the natural sunshine to which both groups were exposed.

All the cows were fed and treated alike in accordance with the routine system of feeding and managing this herd. Daily milk production records were kept and the milk from each cow was tested for butterfat once a month in each trial except the last when it was tested weekly.

Various treatments of the production data obtained during the first year yielded no significant difference in performance of the two groups of cows.

During the second year the irradiated cows were subjected to about three times as much ultraviolet as during the first year. Again no significant difference in production was obtained. A combination of data for the two years showed the average daily production of 4 per cent milk for 34 check cows to be 31.9 pounds, and of 36 irradiated cows 32.4 pounds.

Two additional trials were run in which previous production performance, stage of lactation, etc., rather than agglutination titer, were used as a basis for dividing into groups. The significant analyses of the data obtained were based on paired performance during the experimental period as compared to performance in a preliminary period. The amount of ultraviolet radiation given was much greater than that used in the previous trials and approached the daily equivalent of 10 hours of midsummer sunshine.

In the first of these trials (6 pairs) the irradiated cows produced 0.75 pound of 4 per cent milk more daily than was expected, based on the performance of the control cows during the 91-day experimental period. In the second trial (8 pairs), over a period of 172 days, the irradiated cows produced 0.79 pound of 4 per cent milk more daily than was expected.

It is concluded that the light sources used (S–1 and S–4 lamps) had no effect on milk production.

P7. Studies on Milk and Fat Production of Dairy Cows and Goats as Influenced by Thyroxine.* N. P. RALSTON, C. W. TURNER AND A. C. RAGSDALE, Missouri Agricultural Experiment Station.

The initiation and maintenance of milk secretion has been definitely demonstrated to be influenced by numerous hormones. Thyroxine, a secretion of the thyroid gland, has been shown in recent experiments to augment milk and fat production.

In recent investigations at this station eighteen cows received 90 injections of thyroxine at monthly intervals during their lactation periods. The

* Contribution from the Dept. of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 608.

hormone was injected subcutaneously, in an alkaline solution, at the rate of 15 mg. per day for three successive days during each month. This treatment caused an average increase in milk production of 13.6 per cent, increased the total pounds of fat 22.5 per cent, the per cent of fat 4.75 per cent, the total pounds of solids-not-fat 14.7 per cent, and a slight increase in the per cent of solids-not-fat. The pulse rate increased an average of 7.64 beats per minute, while body temperature remained within normal range.

Milk production reached its maximum on the sixth and seventh days after starting the injection. Milk production then returned gradually to its normal level by the 12th to 14th day. Cows in the second to ninth month of lactation showed a greater response than cows in first, tenth, eleventh and twelfth months of their lactation.

A study of the blood constituents of four goats injected with thyroxine was made in order to note changes of milk precursors during the increased milk production. The goats were given subcutaneously 12 mg. of thyroxine in alkaline solution during the late declining phase of their lactation period. Milk and fat production increased on the average of 67 per cent. The osmotic pressure of the blood serum and the milk indicated no significant change. Blood sugar and blood fat showed a reciprocal relationship. Blood sugar increased during the first part of the experiment while blood fat decreased. During the latter portion of the experiment the opposite trend was shown. The percentage of lactose increased while the percentage of milk fat declined throughout the experiment. Total nitrogen and non-protein-nitrogen declined slightly both in blood and milk during the entire period of two weeks.

There was a definite increase in the respiratory rate with a slight increase of body temperature. Heart rate increased markedly. Systolic blood pressure increased slightly except in cases where very high heart rate was noted.

Prolonged hyperthyroidism in humans have in some cases shown deleterious effects on the cardio-vascular system. Electro-cardiographic records have been taken of goats and cows given thyroxine in order to determine if any ill effects develop. At the present time no cardio-vascular damage has been noted. However, the period of thyroxine injection has been too short to draw definite conclusions. This study is being continued.

P8. Two Methods of Feeding Soybean Fat to Cows and Their Effect on Milk and Butterfat Production and on the Nature of the Butterfat.

N. KENNETH WILLIAMS, C. Y. CANNON AND DWIGHT ESPE, Iowa Agricultural Experiment Station.

In one of a series of experiments on soybean feeding conducted at the Iowa station, two groups of cows were used in a double reversal trial of four week periods, in which the rations of alfalfa hay, corn or sorghum silage and grain were identical except that the soybeans in one grain mixture (1 corn, 1 oats, 2 cracked soybeans) were replaced in the second by the soybean oil meal and soybean oil obtained by processing part of the soybeans at a low temperature so as not to alter the biological value of the protein. The grain was mixed weekly in order to avoid rancidity. Feeding was regulated so that each cow received from the grain about eighty per cent as much fat as she secreted in her milk.

The results obtained were quite marked and consistent with previous observations made at this station. Milk production averaged 7.8 pounds per cow per week more on the "oil" ration. However, without exception, the cows produced more butterfat when the cracked soybeans were fed. The mean advantage of the soybean ration in butterfat percentage was 0.34. From comparison with production during the preliminary period when a "normal" grain ration was fed and from data obtained from previous experiments, it is evident that both types of feeding were responsible for the trends; that is, the feeding of cracked soybeans decreased milk production and increased butterfat production and fat percentage; while the feeding of soybean fat as oil increased milk production but decreased butterfat production and fat percentage.

Changes in live weight of the cows were erratic and non-significant. Chemical and physical measurements were made on the butterfat obtained from periodic group and individual samples of milk. Both rations caused great changes in the distribution of fatty acids as indicated by various constants. However, the "oil" ration caused the most profound changes; during the periods when this ration was fed the iodine and thiocyanogen-iodine values were at their maximum and the Reichert-Meissl and Polenski values at their minimum. Calculation of the content of linoleic (or its optical isomer) and oleic acids (based on iodine and thiocyanogen-iodine values) showed that the oleic acid content was highest during the oil feeding periods and the linoleic acid during the soybean feeding periods. This indicates that the nature of fat metabolism was affected by the method of feeding fat since the total unsaturation in the butterfat was greater when the oil was fed, but the conversion of linoleic acid to more nearly normal constituents of butterfat was more nearly complete.

Churning time of butter was increased during the period of "oil" feeding over the "soybean" periods. Both rations decreased the color of the butterfat compared with normal rations but with the method used to measure color it was not possible to differentiate between their effects. Both rations increased the melting point of the butterfat over that produced by the normal preliminary ration but there was no marked difference in the effects of the two trial rations.

The changes in milk production, per cent butterfat, butterfat production, iodine and thiocyanogen-iodine values, per cent oleic and linoleic acids were subjected to statistical analysis. All were highly significant (one per cent level) or significant (five per cent level).

P9. The Effect of Sulfanilamide Therapy upon the Catalase Activity of the Blood and Milk. A Preliminary Report. P. G. MILLER AND G. W. ANDERSON, South Carolina Agricultural Experiment Station.

Sulfanilamide has been used successfully for the past two years for the treatment of mastitis in the college herd. Recently Locke, Main and Mellon (Science, Dec. 30, 1938) proposed the theory that sulfanilamide was effective in retarding infections due to pneumococcus and hemolytic streptococcus indirectly by sulfanilamide oxidative products formed within the multiplying organisms having sufficient anti-catalase action to permit injurious amounts of hydrogen peroxide to be accumulated.

The action of organisms in a case of streptococcic mastitis is believed to be confined to the udder; so if anti-catalase, as postulated, should be present in the blood stream of an animal having only such an infection, it would necessarily come from the udder. Preliminary work in which sulfanilamide was given to cows emitting streptococci in the milk but not showing clinical symptoms of mastitis have not shown any definite effect on the blood catalase activity. Neither has the milk catalase activity shown any significant trends that can be definitely attributed to sulfanilamide therapy.

Determinations are being made to compare the effect of various conditions upon the catalase activity of the blood of the mammary veins and an ear vein, and of the milk from individual quarters; that is, while the cow is in normal health, during an artificially infected acute case of mastitis on one side of the udder, during sulfanilamide therapy and recovery.

P10. The Effect of Ultraviolet Radiation of Cows On Their Agglutinin Titer for Bangs Disease. B. H. EDGINGTON, W. E. KRAUSS, C. F. MONROE, AND C. C. HAYDEN, Animal Disease Research Laboratories and Department of Dairy Industry, Ohio Agricultural Experiment Station.*

The object of this experiment was to ascertain the effect, if any, that ultraviolet radiation of cows in a Bangs diseased herd might have on the course of the disease in the herd, as determined by monthly agglutination titers of blood and milk from each cow.

The animals used were part of a state-owned Holstein herd in which Bangs disease had existed for several years. The animals were separated on the basis of their age (2–12 years) and agglutinin titers into two groups designated as Control and Irradiated. The cows were classified as non-reactor, questionable and reactor on the basis of the agglutinin titer of their blood. Data were compiled only on those animals that were under observation during the entire 29 months of the experiment. The Control group contained 31 cows and the Irradiated group 24 cows.

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* In cooperation with the State Department of Public Welfare and the General Electric Company, Nela Park, Cleveland, Ohio.

During the first year S-1 sunlamps were used for irradiating the cows of the Irradiated group. It was calculated that the group received during the year the equivalent of approximately 100 hours of midsummer sunshine, in addition to the natural sunshine to which the cows of both groups were exposed. During the second year the cows were exposed to about three times as much ultraviolet as during the first year.

The classification of the animals, based upon their agglutinin blood titers at the beginning and termination of the experiment, showed the following changes: In the Control group, the 11 cows that were originally non-reactors remained non-reactors; 2 of the 9 questionable ones became non-reactors, and 2 of 11 reactors became questionable. In the Irradiated group one of the 7 non-reactor cows became questionable; 5 of the 10 questionable ones became non-reactors, and the 7 reactors remained in that class.

The titer of agglutinins in the milk from different quarters of the same cow was variable and in a few tests was higher than that of the blood collected on the same day. One non-reactor and two questionable cows of the Control group and all the reactors of both groups gave questionable or reactor titers in one or more quarters at least once in the series of tests. No other cow of either group had a titer exceeding that of a non-reactor.

During the period of this experiment 101 pregnancies occurred—56 in the Control and 45 in the Irradiated group. Five of these gestations terminated in abortion; however, *Br. abortus* was demonstrated in but one of the fetuses. These results, plus the fact that but one of the non-reactor cows of both groups aborted, indicates that the intra-herd spread of *Br. abortus* was probably very limited.

This experiment fails to give evidence that the light sources used in this herd had a significant effect on the course of Bangs disease under the conditions of this particular experiment. It should be pointed out, however, that the ultraviolet light employed ranged in wave length from 2800 to 3200 Ångstrom units which does not include the sterilizing range.

P11. A New Routine Extending the Usefulness of the Hotis Test for Mastitis. J. FRANK CONE, U. S. Bureau of Dairy Industry.

Criticism has been directed at the Hotis test because of the number of borderline or "suspicious" reactions. This objection to the test can be overcome by combining it with the microscopic examination of the incubated sample. Quantitative experiments show that the concentration of dye in the Hotis test is not so great that it will delay or retard the growth of mastitis streptococci.

With the new routine, the Hotis test is set up in the usual manner, *i.e.*, adding 9.5 ml. of the sample to 0.5 ml. of 0.5 per cent brom cresol purple solution in a test tube. Immediately a record is made of each tube, showing whether the milk is normal or alkaline in reaction. The tubes are incubated at 37° C. for 20–24 hours and examined for the characteristic canary yellow flakes, with or without the development of acid in the milk. A distinctly positive test is considered complete and examined no further. All "suspicious" or negative samples are then examined microscopically for the presence of long-chain streptococci.

One thousand and ninety-seven individual quarter samples have been examined in this manner and with direct plating on Edward's aesculin blood agar. Of these, 187 samples were found to contain mastitis streptococci. The Hotis test gave 125 positive reactions, all of which were confirmed on Edward's agar. It gave negative reactions on 898 samples, 8 of which showed streptococci in the smear and on the blood-agar plates.

It is believed the additional information obtained with little additional labor involved by combining the tests is a distinct advantage.

P12. The Classification of the Streptococci of Bovine Mastitis.

R. B. Little and F. C. Minett, The Rockefeller Institute for Medical Research, Princeton, N. J., and Royal Veterinary College, Camden Town, London.

The purpose of this note is to call the attention of workers who are interested to a project initiated by one of us (R.B.L.). The necessity for trying to get some orderly arrangement of the streptococci which may be associated with bovine mastitis has long been obvious. Not only is this so on the grounds of bacteriological nomenclature, but, unless investigators in different parts of the world use the same designations, it is difficult or impossible to make those comparisons which are essential for a proper understanding of the subject and in consequence scientific progress is impeded. To give an example. The term "Streptococcus mastitidis" has been used in the past for more than one kind of mastitis streptococcus, whereas in fact it is now known that there are several kinds of streptococci recognized by experienced workers as capable of causing different forms of the disease. Moreover, only one of these forms is known to spread easily in a herd. The subject of classification, therefore, has a clear relation to preventive and clinical veterinary medicine

Briefly, we have decided to ask those who are studying mastitis to communicate with us. Persons resident in the American continent and Japan should write to Dr. Little, while those in Europe and the British Empire, are requested to get into touch with Dr. Minett. Persons who are interested would then be requested to re-examine their cultures (1) by means of a group B standard serum which would be issued (2) by certain relatively simple cultural and biochemical methods. Mimeographed copies of suggested methods for carrying out these tests and forms for recording the results would be sent out at the same time, to be returned as soon as possible to Princeton or to Camden Town, as the case may be. Cultures giving "atypical" reactions should be sent for re-examination to one or other of these centers. Type strains would be issued on request for purposes of comparison. Later on, the results of the enquiry would be circulated to all concerned and then, if the majority of workers agreed, they could be published in summary form, with particular reference to "atypical" strains.

P13. Results of a Long Time Feeding Experiment on Bone Meal vs. No Bone Meal in a Dairy Ration Consisting of Timothy Hay, Corn Silage, and Concentrates.

S. I. Bechdel, P. S. Williams, A. A. Borland, and J. F. Shigley, The Pennsylvania State College.

In order to study the advisability of mineral supplements in the dairy ration, a long time practical experiment was conducted at the Pennsylvania Experiment Station. This experiment was carried out under the conditions that usually exist on a modern practical dairy farm. High grade Holstein cows were used. The whole herd was on pasture (usually fertilized) during the summer months. During the remainder of the year they were housed in a modern barn. The roughages consisted of timothy hay, usually early cut and of high grade, and corn silage. This roughage combination is commonly fed on Pennsylvania dairy farms. The concentrate mixtures fed usually contained about 18 per cent crude protein. Two per cent steamed bone meal was added to the mixtures when the experimental set-up called for it. A total of thirty-three cows with one hundred seventeen complete lactations were studied. Ten of the cows were carried on either the basal ration or the ration containing bone meal until removed from the herd. On twenty-three of the cows the rations (bone meal *vs.* no bone meal) were reversed every lactation. The milk records were corrected to four per cent fat, three time milking, and maturity.

The milk production for the twenty-three cows that were on both rations averaged 8653.9 pounds when they received the basal ration and 9008.1 when they received the ration containing bone meal. This difference appears to be due to a slightly longer lactation (10 days) when the cows received bone meal. The cows receiving the bone meal also produced slightly more milk per pound of grain (3.07 for the basal and 3.134 for the bone meal group) than did the group on the basal ration. One and seventy-three hundredths services were required for conception when the cows received the basal ration and 1.59 services when the cows received bone meal. A few cows that received the bone meal ration for two or more years in succession required only 1.33 services in the second year on bone meal. The calves from cows that received the bone meal weighed on the average three pounds more at birth. No significant differences were observed in the general health of the animals, and the incidence of mastitis or other pathological conditions that might be caused by mineral deficiencies.

The results of this experiment show conclusively that no outstanding deficiency of minerals resulted under the conditions of this experiment when the cows were fed a ration containing no bone meal. These cows were all grazed during the summer months on pastures treated with mineral fertilizers, and this pasture may have helped out in supplying any possible deficiency of minerals.

Although the differences in favor of bone meal are small, the results are consistent enough to make them of real significance. The additional milk produced was sufficient to net a return three to four times the cost of the bone meal.

P14. Making and Feeding Air Dried Hay to Dairy Heifers.

C. E. Wylie and S. A. Hinton, University of Tennessee, J. W. Weaver, Jr., Tennessee Valley Authority.

Many sections of the country have difficulty in curing hay after a good crop is grown. This is due to damage by rain. Then, too, there is considerable loss by shattering of leaves in curing hay in the field. For many years there has been an attempt to improve hay curing by the use of large commercial hay driers requiring heat. At the University of Tennessee there has been developed during the last four years a drier which cures hay in the barn loft without the use of heat.

This new process includes a series of wood ducts on the floor of the hay loft. Air is forced through these ducts and through the hay by the use of a 5 hp. electric motor and a blower which delivers 12,000 cu. ft. of air per minute. The hay, in the case of alfalfa, is allowed to cure about four hours in the field when it will contain about 50 per cent moisture. At this stage it is limp and will not shatter. It is placed in the hay loft using the ordinary hay harvesting machinery. In the loft the forced air brings the moisture down to 20 per cent which is safe for storage.

Analyses of this hay, grading and feeding trials, indicate that it is superior to hay cured in the field. This includes two years of feeding trials with dairy heifers. The growth results were especially favorable the winter of 1938–39.

P15. The Nutritive Value of an Alfalfa Hay Ration Compared with a Mixed Grass and Legume Hay and Grass Silage Ration for Dairy Cattle.

R. E. Hodgson and J. C. Knott, Washington Agricultural Experiment Station, Bureau Dairy Industry Cooperating.

Alfalfa hay grown in one of the irrigated alfalfa sections of central Washington was compared with mixed grass and clover hay and grass silage grown in western Washington as a ration for dairy cows. The alfalfa was fed as the complete ration to one group of cows while the western hay and grass silage (home-grown ration) was fed as the complete ration to another group of cows.

The alfalfa used was Commercial U. S. No. 2 second cutting alfalfa. The home-grown mixed hay was U. S. No. 2 clover timothy and rye grass mix. It was harvested when clover was in about full bloom and the grasses fully headed. The grass silage was harvested in the early head stage. The two roughage rations were fed in amounts slightly in excess of what the cows would consume. In the hay and silage ration 58 per cent of the dry matter consumed was fed in the form of hay.

Duplicate experiments were conducted the first in 1937–38 with nine cows in each group fed for a period of 168 days. The second experiment was conducted in 1938–39 and four cows in each group were fed the two rations for 140 days. At the beginning of each experiment the two groups of cows were as nearly alike as possible in regard to production, age, weight and gestation.

The average protein content of the feeds was alfalfa hay 14.2 per cent, home-grown mixed hay 8.9 per cent and grass silage 12.6 per cent.

On the alfalfa ration the average daily consumption of dry matter was 33.2 pounds in Experiment 1 and 33.4 pounds in Experiment 2, an average of 33.3 pounds. The cows consumed 96 per cent of the dry matter fed. On the home-grown mixed hay and silage ration the average daily consumption of dry matter was 26.1 pounds in Experiment 1 and 26.3 pounds in Experiment 2. This was 78.6 per cent as much dry matter as was consumed by the group of cows receiving alfalfa hay. The cows on this ration consumed 95.1 per cent of the dry matter fed.

On the alfalfa hay ration the cows lost an average of .2 pound live weight per day in Experiment 1 and .4 pound in Experiment 2. On the home-grown mixed hay and silage ration the cows lost an average of .8 pound in Experiment 1 and 1.2 pounds in Experiment 2. The cows receiving alfalfa lost only 33 per cent as much live weight as the cows receiving the home-grown mixed hay and silage ration.

On the alfalfa hay ration the average daily production of four per cent fat corrected milk was 23.9 pounds in Experiment 1 and 24.5 pounds in Experiment 2. On the home-grown mixed hay and silage ration the average daily production was 22.0 pounds per day in Experiment 1 and 23.7 pounds in Experiment 2. This was 94.2 per cent as much milk as was produced by cows receiving the alfalfa. The average test was 3.5 per cent for cows fed alfalfa and 3.3 per cent for the cows fed the home-grown mixed hay and silage ration.

P16. Studies With Alfalfa Hay for Milk Production. I. R. Jones and J. R. Haag, Oregon Agricultural Experiment Station.

An exclusive ration of alfalfa hay allows good dairy cows to produce some 45 to 70 per cent as much milk and butterfat as when fed a normal ration. The production obtained on hay alone is affected by previous feeding and the quality of hay. The decline in production with the advance in lactation takes place at about twice the normal rate and continues rapidly even after the point is reached where the total nutrient intake is well above that supposedly required for the production of some 30 pounds of milk. The level of inorganic blood phosphorus is usually below normal but phosphorus supplements alone do not appear to materially arrest the rapid decline in production.

While it is true that cows cannot consume enough total digestible nutrients in the form of alfalfa hay for very high production, the precipitious decline in lactation is suggestive of a lack of protein stimulation. The deservedly high esteem in which alfalfa hay is held is based on palatability, yield and high protein content rather than on specific evidence of high biological value of its protein. The study deals in part with the influence of limited supplements of a mixture of equal parts of linseed, cottonseed, and peanut meals.

The feeding of as little as 2 or 3 pounds of the protein concentrate mixture at first arrested the rapid decline in production and then allowed it to proceed at a normal rate. These experiments indicate that the protein supplied by alfalfa hay alone may be an important factor contributing to a rapid decline in production. At the present time, similar studies are being conducted with single sources of protein.

P17. Comparative Yields of Louisiana Feed and Pasture Crops. R. H. Lush, Louisiana Agricultural Experiment Station.

Yields of various feed and pasture crops are compared in terms of hay equivalent, total digestible nutrients, calcium and phosphorus per acre where grown under similar conditions at five experiment stations in Louisiana. Coefficients of digestibility and of average composition, where not determined locally on crops other than pasture, are adapted from Morrison's "Feeds and Feeding," 20th Edition.

The highest yields of both hay equivalent and total digestible nutrients were obtained from mixed pasture at all locations except at Hammond, on flat wood land, where lespedeza fertilized with superphosphate out-yielded fertilized pasture in terms of nutrients, calcium and phosphorus, and at Calhoun, hill land, where well fertilized alfalfa was the highest yielding crop where grown, alfalfa or red clover produced the most calcium per acre, and at Calhoun the most phosphorus as well. In other locations fertile mixed pasture produced more phosphorus than any other crop, including the grain crops. This is important in the phosphorus deficient areas. Fertilized pasture at Hammond produced only 39.4 per cent more hay but 80.9 per cent more phosphorus per acre than the same kind of unfertilized pasture. Fertilized lespedeza at the same station produced 2–3 times as much hay and 3–5 times as much phosphorus and calcium as untreated lespedeza.

The differences between good pastures and poor pastures within the state may be greater than between crops grown in the same location, however. The best alluvial land pasture at Baton Rouge produced 9985 pounds air-dried hay containing 5068 pounds total digestible nutrients, 56.8 pounds calcium and 36.4 pounds phosphorus. Unfertilized flat woods pasture 50 miles away produced only 2855 pounds hay, 1231 pounds total digestible nutrients, 10.9 pounds calcium, and 3.0 pounds of phosphorus per acre. While the calcium-phosphorus ratio above varies from 1.5 : 1 to 3.6 : 1 for the extreme kinds of mixed pasture, it is probably more desirable for cattle feeding than in other feed crops. A yield of 92.7 bushels corn per acre would supply as much total digestible nutrients as the fertile pasture above but only 38 per cent as much phosphorus and less than one per cent as much calcium.

Hay equivalent per acre is not a satisfactory comparative measure as the total digestible nutrients varied from 43.1 per cent on prairie soil, Crowley, to 54.3 per cent for fertile hill pasture, Calhoun. Alfalfa hay equivalent makes the above corrections for nutrients but assumes too much calcium and too little phosphorus for mixed pastures of Louisiana. Total digestible nutrients per acre is less subject to the above criticism, especially if mineral content is determined. Feed replacement values, dependent on feed prices and level of production, are also in error. Milk production, four per cent fat-corrected, per acre is probably more accurate.

This paper emphasizes the fact that improved pastures, regardless of the method of comparison, produce large amounts of well balanced dairy feed per acre in Louisiana as well as in other states. While costs are not included, it is believed that the pasture was a more economical source of feed as well as fitting better into a land conservation system.

P18. Pasture Yields as Measured by Clip Plots and by Grazing Dairy Cows. P. M. Brandt and H. P. Ewalt, Oregon Agricultural Experiment Station.

The yield from an area of 8.72 acres of mixed grass and Ladino clover pasture being grazed for the fifth consecutive season was simultaneously measured by the clip plot and grazing methods. The area was divided into four fields of approximately 2 acres each. The grazing practice was to rotate the cows often enough to enable them to graze to their maximum capacity. Under this system of management, each field was grazed 13 or 14 times during the season of 189 days.

The season was started with 15 cows, of which 8 were Holsteins, 3 were Ayrshires and 4 were Jerseys. As the season progressed, 3 cows were removed from the area to insure ample grazing for the others.

The carrying capacity for the season of 189 days was 12.43 cows or a total of 2351 cow days. The average initial weight per cow was 1020 pounds with an average daily gain for the season of 0.57 pounds. The average daily production per cow was 44.1 pounds milk and 1.45 pounds butterfat. According to the Morrison standard, the total digestible nutrient requirement was 23.44 pounds per cow daily and the amount supplied in the barn by concentrates was 8.13 pounds per cow daily. By difference it has been calculated that the pasture supplied 15.26 pounds of total digestible nutrients per cow daily or a total of 35,958 pounds for the 2351 cow days. The cows thus received 65.3 per cent of their feed requirements from the pasture.

Four one-hundred square-foot areas in each field were protected from grazing and clipped at the end of each grazing period. The yield of dry matter by the clip plot method from the 8.72 acres for the season was 54,194 pounds. Assuming the dry matter to contain 70 per cent of total digestible nutrients, the digestible nutrient yield has been calculated to be 37,936 pounds.

From the data given, the grazing method yielded 1978 pounds, or about 5 per cent, of total digestible nutrients less than the clip plot method. The difference of 5 per cent was obtained under conditions in which the barn was located adjacent to the pastures. In a previous study where the cows walked 4 miles daily to and from pasture, we obtained a difference of some 20 to 25 per cent in favor of the clip plot method.

It appears that under the conditions of this study, the clip plot method quite satisfactorily measured pasture yields.

P19. The Chemical Composition and Nutrient Yield of Pasture Grass Grazed at Successive Intervals During the Season. R. E. Hodgson and J. C. Knott, U. S. Bureau Dairy Industry and Washington Agricultural Experiment Station Cooperating.

The chemical composition, dry matter and nutrient yields have been determined on pasture for successive grazing intervals during each of the past three years. The five acre pasture was located on muck soil and was seeded in 1929. The predominating flora were Kentucky blue grass, English rye grass, meadow fiscue and a small amount of white clover. During each grazing period 20 cages were located over the pasture to protect the grass from grazing. After each grazing the grass was cut and the dry matter yields determined. The dry matter was composited and a representative sample analyzed for its nutrient content. Each grazing was started when the grass was in about the same stage of maturity. Cows grazed the pasture six times the first year, five times the second year and four times the third year. The days between the end of each grazing were considered the growing period for the grass.

Each year the dry matter content of the pasture grass was highest in midsummer when the temperature was highest and the precipitation lowest. The protein content of the dry matter increased progressively as the seasons advanced, the range being from 12.7 per cent to 22.7 per cent the first year; 13.2 per cent to 23.4 per cent the second year and 12.1 per cent to 19.6 per cent the third year. The crude fiber and nitrogen-free extract content of the dry matter decreased progressively as the seasons advanced. The ether extract content increased slightly as the seasons advanced in the first and third year, whereas, in the second year it was higher during mid-season. The calcium content of the dry matter was higher during the drier and hotter part of the summer. The phosphorus content varied only slightly between successive grazing in each of the three years and no definite trend was observed.

The yields of dry matter, crude fiber and nitrogen-free extract decreased markedly as the seasons advanced. The ether extract, calcium and phosphorus decreased much less rapidly with advancement in the season, while because of the higher protein content of the dry matter, the yields of protein in 1936, particularly, were as great at the end of the season as earlier. The variation from year to year in total yield of dry matter and nutrients did not necessarily vary with total precipitation during the growing period (considered from April 10 to October 20).

P20. Some Relations of the Roughage Intake to the Composition of Milk. E. B. POWELL, Ralston Purina Company, St. Louis, Mo.

During the past thirteen years the Purina Mills Experimental Farm has conducted a series of experiments to determine some of the relations between roughage consumption and variations in milk composition. This series of tests has involved 37 cows, mostly Holsteins, for a total of 85 lactations.

These experiments have shown that the fat content of milk was varied at will, as much as 60 per cent, by regulating the physical characteristics and total intake of the roughage part of the ration. This was done at will and without materially changing the quantity of milk produced.

The results further show that a cow may be maintained at the low fat level for at least three complete lactations without any apparent physical injury and can then be brought back to the normal fat percentage permanently by changing the physical characteristics of the roughage received and/or the amount of roughage received. The solids-not-fat also vary in the same general manner as the fat.

There is apparently a positive correlation between the activities of the rumen and the composition of the milk produced. We believe that the results of this series of tests show conclusively that the ration the cow receives does influence the per cent of fat in the milk, also the per cent of solids-not-fat and that these changes persist as long as the type and composition of ration is continued unchanged.

Some later work, still in progress at the Purina Mills Experimental Farm, indicates that the physical condition of the roughage and its quantity are not the only factors involved, but that the physical condition of the cow at freshening time and the composition of the concentrate used may have some bearing on the results secured.

In order to determine definitely whether or not rumination is an essential process for the production of milk of normal composition, we have removed the rumen from some dairy heifers at the Experimental Farm. These heifers will be grown out to maturity and will be used for further study on this problem.

P21. The Influence of Certain Rations and Management Practices on the Rate of Growth and Production of Holstein-Friesian Heifers.* H. A. Herman and A. C. Ragsdale, Missouri Agricultural Experiment Station.

This study is concerned with the effects of feed intake and the length of the growth cycle on the size and maturity of Holstein heifers at breeding and freshening time. The effects of rapid growth, high protein diets, and early breeding as reflected in milk production is included as a part of the investigation. This paper summarizes to date the influence of three rations on the rate of growth of Holstein heifers. The rations used consist of (1) milk and alfalfa hay supplemented only with salt and steamed bone meal; (2) a ration called "rapid growth ration" rich in vitamins and minerals and containing 27 per cent crude protein of high biological value; and (3) the rapid growth ration supplemented with 10 per cent of dehydrated cereal grasses. In addition the heifers receiving rations 2 and 3 were allowed 10 to 20 pounds of whole milk (3½ per cent fat) daily. The calves receiving Ration I were fed skimmilk after 9 weeks of age. All dry rations were fed *ad libitum*. Growth was measured by body weight and height at withers and compared to the "normal" as set forth in Missouri Agricultural Experiment Station Bulletins 336 and 338.

The heifers receiving Ration I (chopped alfalfa and skimmed milk) were 13.8 per cent below normal in body weight and 3.8 per cent below normal wither height at 2 years of age. The average freshening age was 28 months. This group was fed *only roughage* consisting of alfalfa hay, corn silage, and limited pasture, and produced an average of 6602 pounds of milk and 256.3 pounds of butterfat the first lactation, which averaged 333 days. A group of normally raised heifers of similar breeding fed the same roughages and grain at the rate of 1 pound for each 4 pounds of milk they produced, averaged 10,198 pounds of milk, 371 pounds of butterfat in 330 days, age 29 months. The sole roughage group averaged 69 per cent as much butterfat as the control group. During the second lactation grain is being fed at the rate of 1 pound for each 6 pounds of milk produced daily. An increase in milk and fat production of approximately 35 to 40 per cent above that obtained on a roughage diet is indicated. Part of this increase of course is attributed to advancing age. The heifers in this group have grown slowly since freshening as 2 year olds. At 3 years of age they averaged 1033 pounds in weight (11 per cent below the standard and were 5 per cent below normal in wither height). They have, however, been unusually hearty eaters, regular breeders, and have suffered no udder troubles.

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* Contribution from the Dept. of Dairy Husbandry, Mo. Agricultural Experiment Station, Journal Series No. 610.

The heifers fed rations 2 and 3 were similar in growth and at 12 months of age were 30 per cent above normal in weight and 4.7 per cent higher at the withers. Since at this age the body weight and size closely approximated an 18 months old heifer, the heifers in this group were bred so as to freshen from 20 to 24 months of age. The average freshening age was 22½ months. In spite of the loss of weight following calving the heifers in this group averaged 1155 pounds at 24 months, 9 per cent above the growth standard and were 1 inch higher at the withers than normal. In production their showing has not been spectacular, the group of five has averaged 6488 pounds of milk, 245.4 pounds of butterfat in 271 days, age 22½ months. They have been fed the regular ration of sorgo or corn silage, alfalfa hay, pasture, and grain mix as is normally alloted cows in the University herd. Since freshening their growth has continued at a normal rate of gain.

From the standpoint of inherent ability the heifers should produce as well as the normal group, but it is obvious they will fall below this level. This factor, aside from the fact that more animals are needed in the experiment, may possibly be contributed to the fact that the heifers were not more mature at freshening. There is also the possibility advanced by many breeders that too heavy feeding of the heifer results in a mediocre producer as a cow. This problem is being investigated further.

P22. Vitamin A and Carotene in Relation to Growth and Well Being of Heifers.

R. E. WARD AND S. I. BECHDEL, The Pennsylvania State College.

Three feeding trials were conducted with dairy heifers fed hays of low carotene content over the winter feeding period. These trials were designed to show any effect that increasing the carotene or vitamin A intake would have on the growth and well being of the heifers. Timothy hay of low grade with only 3 to 7 micrograms of carotene per gram and concentrate mixtures low in vitamin A were used as basal rations. The majority of heifers were on pasture during the summers prior to and subsequent to the feeding trials.

In the first trial twelve heifers were divided into two evenly balanced groups and fed for 150 days. One group received two pounds of alfalfa molasses silage daily per head and the other group received 0.3 pound of soybean oil meal to make the rations comparable. The heifers receiving the soybean oil meal gained an average of 13.2 pounds more per head during the experimental period than those fed the alfalfa molasses silage.

In the second trial conducted over a period of 149 days four heifers received the basal ration only while four similar heifers received 4.5 grams of fortified cod liver oil per head daily in addition. These heifers were younger and had but limited access to pasture the previous summer. The group receiving the fortified cod liver oil made 6.3 pounds per head better gains than the group receiving the basal ration only.

In the third trial seventeen heifers were fed over a 72-day period. One group was fed a fifteen pound daily allowance of alfalfa molasses silage to each animal. The other group was fed similarly a 15 pound daily allowance of corn silage. The heifers receiving the alfalfa silage made 2.5 pounds better daily gain per head. This gain is probably due to the higher protein intake rather than to the increase in carotene.

A study of the breeding data on the heifers used in these trials show no appreciable differences in the number of services required for conception or on the health of the calves. The general health of the heifers appeared to be similar in all groups. Under the conditions of these trials it appears that carotene or vitamin A supplements will promote little or no increased growth or improved well being over a winter feeding period if hay of low quality is fed when the heifers have been on pasture during the previous summer.

P23. Carotene Changes in Natural Cured, Artificially Dried and Ensiled Roughages.

O. M. CAMBURN, H. B. ELLENBERGER AND G. C. CROOKS, Vermont Agricultural Experiment Station.

Alfalfa, red clover and timothy crops of 1938 were preserved by ensiling alone, with molasses and with phosphoric acid, and by making sun cured and artificially dried hays. Each lot of silage was sampled when ensiled and again when removed for feeding. The hays were sampled when cut, when stored, and after 60, 120 and 180 days of storage. The carotene content of each sample was determined.

When cut, the second crop alfalfa carried twice as much carotene, 294 gamma per gram of dry matter, as did the first cutting alfalfa, timothy, or clover.

Carotene was preserved most satisfactorily by artificial drying. Little was lost in the drying process and there was about 70 per cent retention after six months storage. The silages retained nearly twice as much carotene as did the sun cured hays, 47 and 24 per cents respectively. One method of ensiling was not outstandingly better than another for retaining carotene.

After about six months of storage the sun cured hays had lost three-fourths, the silages one-half and the artificially dried hays about one-fourth of the carotene content of the green crops.

P24. Some Effects of Feeding Vitamin A and Carotene to Cows, on the Composition of their Milk and Blood. C. H. WHITNAH, W. J. PETERSON, H. W. CAVE, AND F. W. ATKESON, Kansas Agricultural Experimental Station.

In the first test twenty cows of four dairy breeds were studied. Blood from each cow was tested for carotene. A three-day composite sample of milk from each cow was tested for fat, lactose, protein, ash, calcium, phosphorus, and the color, refractive index, and melting point of the fat. The average daily yield for the three test days was also determined. After these tests, nine of the cows were fed a vitamin A concentrate for twenty days. The other cows were used as controls. The tests on blood and milk were repeated at the end of this period. A carotene concentrate was then substituted for ten days for the vitamin A supplement to the first group of cows. The milk constituents were again analyzed at the end of this period.

In a second test, three groups of ten cows each were matched as to breed and stage of lactation. The second and third groups were also matched as to milk flavor. They are the cows discussed in the following paper. Cows of the first group were fed another vitamin A concentrate similar to that used in Test 1. Cows of the second group were fed a more concentrated fish oil at a higher level, and mixed with the feed fresh daily. Cows of the third group were used as controls. After this feeding test had run twenty days, the daily carotene intake of each cow in all groups was increased about 0.45 gram by a change from low quality alfalfa to high quality alfalfa. In addition, each cow of group 2 was fed 0.30 gram of carotene as the concentrate used in Test 1. Four days were used as a transition period and the test was then continued ten days. Milk and blood tests were completed just before the feeding test and after ten, twenty, and thirty-four days.

Vitamin A fed as the fresh oil apparently increased the fat content of milk, while the oil which had been stored as an emulsion lowered the fat test. All the oil supplements increased the refractive index of the butterfat, but none of them greatly changed the melting point.

P25. The Effect of Feeding Vitamin A and Carotene to Cows on the Flavor of Their Milk. H. W. CAVE, W. H. MARTIN AND G. H. BECK, Kansas Agricultural Experiment Station.

The purpose of this experiment was to study the effect of feeding vitamin A and carotene to a selected group of cows on the flavor of the milk which they produced. Twenty cows representing four breeds were selected and divided into two lots. Lot I served as a control and received the regular ration plus 10 grams of molasses daily per cow. Lot II was fed 300,000 I.U. of vitamin A daily per cow in 0.6 gram of oil prepared by the Hickman process. Each cow's allowance of oil was homogenized in 10 grams of molasses before feeding.

After twenty days of feeding to Lot II the vitamin A supplement was discontinued and a carotene concentrate containing one-sixth gram of carotene per pound was fed. For the first four days 0.2 pound per cow daily was fed. This was then increased to 2 pounds daily and was continued at this level for 10 days.

Samples of the fresh milk, three day old milk, and three day old pasteurized milk with and without added copper (0.5 p.p.m.) were scored by three judges at the start and at five day intervals throughout the experiment.

The mean scores of the fresh milk for Lot II at the start of the experiment, at the end of 20 days of vitamin A feeding, and at the end of 14 days of carotene feeding were 21.8, 21.8 and 22.25 respectively; and for the control cows were 21.1, 20.5 and 21.1.

The mean scores of the stored milk from Lot II for each of the three observation periods were 18.7, 19.65 and 21.25 respectively; and for the control group were 19.5, 19.4 and 19.45.

The mean scores for the stored pasteurized milk from Lot II were for the three observation periods 20.2, 20.5 and 21.2 respectively; and for the control cows were 19.8, 19.4 and 20.1.

In Lot II the average scores of the stored pasteurized milk to which copper had been added were, for the three observation periods, 15.0, 17.9 and 18.8 respectively; and for the control group were 16.1, 15.4 and 16.4.

Special attention was given to the development of oxidized flavor in the milk from the two groups of cows. At the start of the experiment 30 per cent of the cows in the fed group and 20 per cent of the cows in the control group gave milk that developed an oxidized flavor. The feeding of vitamin A did not change materially the frequency with which the oxidized flavor occurred in the milk of Lot II cows. After the carotene was fed none of these cows produced milk which developed oxidized flavor, while 20 per cent of the cows in the control group continued to produce the off flavored milk.

The feeding of 300,000 I.U. of vitamin A per cow daily did not materially improve the flavor of the milk, neither did it protect the milk against the development of an oxidized flavor. However, in the milk to which copper had been added there was some reduction in the intensity of the oxidized flavor. When carotene was fed there was a noticeable improvement in flavor and the occurrence of oxidized flavor was entirely eliminated in the fresh and in the stored untreated milk.

P26. Anatomical and Physiological Changes in the Pituitary Gland in Vitamin A Deficiency. T. S. Sutton, Ohio State University.

The pituitary glands of white rats were studied in an attempt to determine the *modus operandi* of vitamin-A deficiency in the production of sterility.

The application of histological methods showed a significant increase in the "basophilic" cells in the hypophyses of vitamin-A deficient rats of both sexes. This increase in basophil count was greatest in the males. The anatomical changes observed were of sufficient magnitude that it was felt that an assay for gonadotropic substance should show differences in physiological activity.

A sensitive technique for the assay of gonadotropic activity was devised. By employing this technique, anterior pituitaries were assayed for gonadotropic activity. The data obtained show that pituitary substance from normal males has greater gonadotropic activity than that from normal females. In vitamin-A deficiency, the gonadotropic hormone content of the pituitary is increased. This increase in potency is greater in the male than in the female. The sensitivity of the technique is illustrated by the fact that 0.14 mg. of anterior pituitary substance gives a measurable stimulation.

This study provides evidence that a vitamin-A deficient diet causes direct damage to the gonad, the increase in basophilic cells and gonadotropic potency represents compensatory changes in the anterior pituitary.

P27. A Field Study of the Influence of Restricted Winter Rations on the Blood Calcium, Phosphorus and Carotene of Dairy Cattle.

W. H. RIDDELL, F. W. ATKESON, W. J. PETERSON AND W. W. THOMPSON, Kansas Agricultural Experiment Station.

The cumulative effect of successive dry years together with rations from restricted plant sources has been reflected in the thrift, yield and reproduction of many herds in the Central Plains area. This past year in Kansas there was little or no pasture available since summer. In normal years wheat pasture is usually available in the state throughout the fall and part of the winter. With the supply of alfalfa hay also limited in the state, a majority of farms depended largely on carbonaceous roughages, principally the sorghum fodders. Furthermore, on many of these farms the amount of protein supplement fed was limited or none at all.

Research elsewhere has shown that forage crops are lower in available phosphorus in a dry year. Also the carotene content of dry roughages is notably less than in pasture or green leafy hays. The principal object of this field study was to determine the effect that lack of pasture and feeding low grade roughage had on the blood picture, particularly with reference to the calcium, phosphorus and carotene levels.

One hundred fifteen samples were collected on the 36 farms in representative sections of the eastern two-thirds of Kansas. These farms were typical of feeding practices followed in many herds in Kansas this past fall and winter. Calcium, phosphorus and carotene were determined on the blood serum less than 24 hours old.

The following average values were obtained:

Calcium	10.55	mgms./100 cc.,	range	8.8 –13.2	mgms.
Phosphorus	5.35	" " ",	"	2.0 –10.3	"
Carotene	0.394	" " ",	"	.018– 1.57	"

Except where symptoms of abnormal appetite had been reported recently, the condition of the animal was no index of the mineral level in the blood. In several instances where a herd because of general lack of thrift was thought to be an excellent prospect for low mineral values, the reverse was found to be true. Apparently the level of production in such herds was at such a low level as to constitute little drain on the mineral reserves of the animal.

On the other hand the quality of the ration was directly reflected in the blood carotene. Generally speaking, the animal's condition was a good index of its carotene intake.

Although many of the rations were deficient in protein and in some cases total nutrients, the outstanding finding was the extensive need for vitamin A as reflected by abnormally low blood carotene values.

P28. Rickets in Calves, A Regional Problem. A. H. Kuhlman and W. D. Gallup, Oklahoma A. & M. College.

The manner of supplying vitamin D in the ration of dairy calves for most efficient utilization of calcium and phosphorus and the prevention of rickets has been the subject of recent investigation. Much of the early work on this subject was done with calves maintained in semi-darkness and receiving a basal ration of high Ca or P content. The experimental conditions imposed were favorable to the development of rickets and may not be considered as normal.

In view of the abundance of sunshine in certain of the southern states, the need in these localities of specific vitamin D supplements in calf rations has not been emphasized. It is recognized that in such regions under ordinary conditions of feeding and management, calves probably receive sufficient amounts of vitamin D to meet their daily requirements. Only after restriction of calves to a simplified ration with limited exposure to sunlight have the symptoms of vitamin D deficiency and rickets appeared. In studies at the Oklahoma station, calves have been raised on a ration of cottonseed meal and molasses beet pulp with supplements of bone meal and vitamin A. Unless exposed to direct sunlight or given vitamin D supplements, these calves developed rickets of varying degrees of severity. Prairie hay when fed ad libitum in place of beet pulp has protected the calves against rickets.

The calves from birth were raised in individual pens in a wing of the dairy barn which has liberal window exposures on the east, south, and west.

During fair weather, large double doors on the south and windows on the east and west were kept open during the day. Determinations of blood Ca and P were made at regular intervals to follow the course of rickets. The calves received outside exposure or vitamin D supplements only after a rachitic condition was established. The vitamin D supplements employed were "aerated" and plain codliver oil, viosterol, and prairie hay.

Blood calcium values dropped from 9-12 Mg. per 100 Ml. of plasma to 8 Mg. preceding the addition of vitamin D supplement. Blood P values were in many instances unaltered. External symptoms of rickets were evident.

Normal blood calcium and phosphorus values were restored within a week after outside exposure to sunlight even during the winter months or following the addition of vitamin D supplements. No cases of rickets developed among the calves fed prairie hay.

Detailed results in mimeographed form are available.

P29. Further Studies on the Vitamin D Deficiency of Mature Dairy Cows. G. C. Wallis, So. Dak. Experiment Station.

Experimental studies establishing the fact that liberal-milking dairy cows have a definite vitamin D requirement which must be satisfied if the animal's health and vigor are to be maintained have been previously reported from this station. Continuous investigational work in this general field has revealed additional information which will be discussed in this paper in a preliminary way.

1.—The possibility of determining the concentration of vitamin D in the blood plasma and using this information to indicate the degree of deficiency or sufficiency of this factor in the animal's body has been investigated. The results give promise that this technique may prove to be a very useful and sensitive device to use in connection with vitamin D deficiency investigations and other studies such as the vitamin D requirements of animals and the value of various vitamin D carriers as sources of this factor. For example, cows receiving an alfalfa hay and grain ration have shown about 1.25 to 1.50 International Units of vitamin D per cc of blood plasma, whereas cows that were markedly deficient in vitamin D as measured by other criteria showed only traces of vitamin D in the blood plasma.

2.—The rate at which milk is produced is an important factor in determining the rapidity with which a vitamin D deficiency condition is produced. Some cows on this experiment have produced up to 60 or 65 pounds of milk per day and have shown definite symptoms of a vitamin D deficiency in the comparatively short time of 3 or 4 months, whereas cows giving less milk have become depleted only after the lactation was nearly completed. To represent the extreme condition, a dry, barren cow is being carried along under vitamin D deficient conditions.

3.—The antirachitic action of late August and September sunshine was found to be very effective in overcoming the vitamin D deficient condition of one of the animals on this experiment. This is significant in view of the fact that one group of workers who used an entirely different approach to the problem, have previously reported that milking cows were unable to utilize the antirachitic action of sunshine.

4.—Observations have also been made on such other problems as the ability of an increased calcium and phosphorus intake to relieve vitamin D deficiency conditions, the value of alfalfa hay as a curative agent, the quantitative requirements of cows for vitamin D, the possibility of vitamin D reserves being lost through excretion into the intestinal tract, and the mineralization of the skeleton of calves born to vitamin D deficient cows as indicated by chemical analysis of the sixth ribs.

P30. Vitamin E Studies with Male Rats. A. O. Shaw, Ella Woods and J. O. English, Idaho Agricultural Experiment Station.

In an attempt to determine the relationship of vitamin E containing feeds to fertility in males, studies were carried on with male rats in the nutrition laboratory at the Idaho Agricultural Experiment Station.

Young male rats when weaned at 21 days of age were placed on the Evans and Burr E-low diet or on this diet plus the supplement to be studied.

When the male rats were not less than 60 days of age, an estrous female of proved fertility was offered each male at approximately weekly intervals. On the following morning residual vaginal spermatozoa were sought in these females and in all cases of sexual congress the fate of the gestation was followed. After three consecutive matings in which sexual congress was evident but no gestation occurred, a different female was used in order to keep the responsibility of failure with the male. The females were kept on the laboratory stock ration both before and after the matings.

The male rats kept on the vitamin E-low diet unsupplemented grew well for 4 or 5 months after which growth continued at a much slower rate and the animals developed a generally disheveled appearance. Forty-six young from eight fertile matings resulted from 13 animals in 232 positive matings.

The results from feeding pea germ meal in amounts up to 3 grams per rat per day, whether used to regenerate fertility at 60, 70, or 80 days of age or used to develop it by feeding from 21 days of age, were no better than from the E-low diet only.

Male rats were fed wheat germ oil in doses ranging from 2 drops per week to 20 drops per day. Those animals which received wheat germ oil up to one drop per day for 7 days a week showed no improvement in fertility over those on the E-low diet only.

Six rats receiving 5 drops of wheat germ oil per day showed 18.8 per cent fertile matings out of 101 trials and 5 rats receiving 10 drops of wheat germ oil daily showed 26 per cent out of 76 trials. However, 3 animals fed 20 drops of the same oil per day showed only 13 per cent fertile matings out of 53 trials.

Histological studies of representative animals from these groups are being made.

P31. Are Urea and Other Simple Nitrogen Compounds Useful in Dairy Cattle Feeding? E. B. HART, G. BOHSTEDT, H. J. DEOBALD, M. I. WEGNER, University of Wisconsin.

The utilization of simple forms of nitrogen in animal nutrition, particularly by ruminants, has been of interest to European more than American investigators. At the University of Wisconsin urea and ammonium bicarbonate have been under investigation the past two years.

The basal ration mixtures for dairy calves in two experiments, each lasting nearly one year, were made up of ground yellow corn, ground timothy hay, starch, corn molasses, steamed bone meal, iodized salt, and cod-liver oil. This basal ration furnished a little less than 6 per cent total protein (N × 6.25) and, therefore, too little for normal growth. Other rations had casein added to raise the protein content of the mixture to 18 per cent. To still other rations, urea or ammonium bicarbonate was added to raise the nitrogen to the same level as that of the casein ration. Urea was also fed at two lower levels. It thus supplied from about 43 to 70 per cent of the total nitrogen of the several rations.

The actual additions to each 100 pounds of the basal ration mixtures were 1.4 pound, 2.8 pounds, and 4.3 pounds urea; 11.1 pounds ammonium bicarbonate; and 11 pounds casein. The plan was to equalize the feed intake to the lowest individual consumption.

Growth on the ration containing the lower level of urea was nearly as rapid as on the casein ration and considerably more rapid than on the higher levels of urea or ammonium bicarbonate. The higher levels of urea proved to be diuretic in their effect. The simple nitrogen compounds were utilized under all conditions although not to the same extent as the protein casein.

Growth records and analyses of blood and other body tissues proved that the simple forms of nitrogen were built into body protein. Only the high urea (diuretic) ration resulted in hypertrophy of kidneys. Those of calves on the low urea ration were entirely normal.

Samples of rumen content of 3 calves fed low urea, high urea, and casein, showed a pH of 7.8, 7.6, and 7.8, respectively. Bacterial cultures revealed no essential qualitative difference.

Where in rations of other ruminants urea used as the only source of nitrogen has resulted in practically no storage of nitrogen, it appears that urea nitrogen may be utilized to a considerable degree when making up only part, in this case about 40 per cent of the total nitrogen of the ration. Apparently the urea or ammonium bicarbonate nitrogen is first converted into bacterial protein through the intervention of paunch organisms and subsequently digested as protein in the true stomach and intestines.

Some of the practical aspects of the problem are the utilization of ammonium compounds and other simple forms of nitrogen frequently found in legume silage, and the considerable proportion of non-protein nitrogen found in heavily nitrogen fertilized pasture.

P32. The Relation of the Rate of Disappearance of Milk Curd from the Calf's Abomasum to the Apparent Digestibility of its Protein.

H. C. Dickey, Dwight Espe and C. Y. Cannon, Iowa Agricultural Experiment Station.

It has usually been assumed in the studies of the digestibility of milk that the more rapid, and possibly, easier passage of the curd from the stomach indicated a superior and more highly digestible milk. The present work is an attempt to determine the effect of the rate of evacuation of the stomach on the coefficient of digestibility of the protein in the ration. Fat-free milk (separator skim milk) from a selected group of Jersey cows was used in each trial. In the first series of experiments the time for the unaltered hard curd skim milk to pass through the entire intestinal tract of young calves was determined by means of a feces marker (carmine). Soft curd skim milk was fed during alternate trials and comparable data obtained. The soft curd milk was prepared by preheating the hard curd milk to 180° F. (82° C.), cooling to 150° F. (66° C.) and homogenizing at 2500 pounds pressure. Curd tension was determined on each batch of milk used. The average curd tension of the hard curd milk was 142 grams, measured by a modification of the Hill method, while the soft curd milk had an average curd tension of 40 grams. Throughout each feeding trial, water, minerals, and cod liver oil were fed in appropriate amounts in addition to the milk.

The milk being tested was fed for a period of 32 hours before the feces marker was given. The results showed that there was no significant difference in the time required for the carmine to make its appearance in the feces when given with the hard curd skim milk as compared with the soft curd skim milk. However, a statistically significant correlation was obtained which showed that as the pounds of milk fed were increased, the number of hours required for the passage of the milk through the gastro-intestinal tract was decreased.

A series of trials, using calves prepared with rumen fistulas, tested, in alternate trials, the rate of evacuation of the two types of curd from the abomasum. The milk, warmed to 37° C., was fed, a liter at a time, through a ¼-inch rubber stomach tube inserted into the abomasum of the calves by way of the rumen fistula and the reticulo-omasal orifice.

It required an average of 9½ hours for the curd from one liter of high tension milk to be liquefied and escape from the abomasum, while that from the soft curd milk required 6¾ hours.

Seven pairs of rats, paired according to age, sex, litter, weight, and amount of milk consumed, were used to determine the coefficient of digestibility of the proteins of the hard and soft curd milks. The seven-day digestion trials, preceded by a three-day preliminary period, showed little difference in the digestibility of the two milks. Trials are now under way with calves to determine the coefficient of digestibility of the proteins in these two types of milk.

P33. Factors Affecting the Reactions of the Esophageal Groove of Dairy Calves. GEORGE H. WISE, South Carolina Agricultural Experiment Station.

A preliminary study, by visual inspection and by palpation of the esophageal groove of fistulated calves, has been made in an effort to discover some of the factors affecting the reaction of this groove. In accord with observations of other investigators consumption of milk, either by nursing or by drinking from open pail, caused the groove to contract and to function as a conduit from cardia to omasum. Filling the rumen with milk via the fistula did not affect the tonicity of the groove, indicating that the reflex does not originate at the distal extremiy of the esophagus.

Drinking tap water usually stimulated the esophageal groove to functional activity in the calf less than three or four weeks of age, after which the response began to subside finally becoming nil. The consumption of a grain mixture, chopped alfalfa hay, chopped *Lespedeza sericea* hay, steamed bone meal, common salt and cod liver oil, respectively, failed to initiate any reaction on the part of the groove.

Generally, the act of sucking stimulated closure of the esophageal groove as evidenced by its response in calves sucking a clean nipple. Thus in nursing both the sucking and the milk apparently stimulated the groove to activity.

In the class of psychic responses may be listed the effects of sight of milk at feeding time and fright immediately preceding feeding. In a calf about a month of age, a view of the milk caused the muscularis of the groove to contract strongly. As the calf become older, the response waned. On the contrary, as noted in only one calf, fright shortly before feeding milk prevented the groove from responding to the stimulus of milk. This was consistently repeated in the one calf but could not be duplicated in any one of six other calves.

P34. Artificial Insemination of Dairy Cows.* H. A. Herman and A. C. Ragsdale, Missouri Agricultural Experiment Station.

Artificial breeding of dairy cows is being practiced at present in nearly one hundred Missouri herds. The greater portion of these herds are included in three cooperative breeding organizations, although several large breeding establishments are using the method to a good advantage. In connection with this program it has been possible to amass some data on the relative efficiency of natural and artificial breeding as well as factors affecting the fertility of stored semen.

Semen collection has been largely by means of the artificial vagina, but in several instances where crippled bulls have been concerned the collections have been made by massaging the ampullae. Most of the semen has been used the same day it was collected, but in connection with some phases of the project the semen has been cooled and stored at 40–45° F.

The University of Missouri herd has been divided into two groups, one-half the cows being bred artificially and the other half naturally. A study of breeding efficiency in this herd covering the past 25 years, and entailing the use of 21 bulls, reveals that 1197 services were required to produce 785 calves, or 1.5 services per conception. This is considerably lower than the average reported by many investigators. Many of the bulls were proved sires from 9 to 15 years of age, but the herd has always been remarkably free of vaginitis and Bang's disease. The bulls ranged from 1.07 to 2.00 services per calf.

In the case of 65 cows in the University herd successfully settled since February, 1938, 32 have been artificially bred. Four of these were considered shy breeders and had been bred from two to four times to the bull before artificial insemination was attempted. The average insemination, by artificial means, required for conception by the cows considered regular breeders was 1.4. The 36 cows naturally bred required 1.56 services. The four difficult breeders were finally settled by artificial means and required from two to four services. These were usually inseminated twice each estrus period, the first insemination being made about the middle of the heat period, and the second 6 to 10 hours later.

In cooperation with the Farm Security Administration, Hughesville, Missouri, an artificial breeding project involving 32 farms and approximately 350 Jersey cows has been in progress the past year. Approximately 250 cows have been artificially bred with an average of 1.8 services. Semen transported 75 miles and held from 3 to 6 hours before use gave a much lower efficiency than semen freshly drawn and used within 1 to 2 hours. The sires used were all highly fertile and were producing spermatozoa of normal morphology and in great concentration.

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* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 609.

Semen stored 18 days at 40 to 50 degrees F. showed approximately 90 per cent motility, but it has thus far been impossible to secure conception with semen stored more than 48 hours and in these instances only two out of seven cows thus bred have been pronounced safe in calf. Studies on this phase of the problem are being continued.

P35. The Inheritance of the Condition for a White Head in Holstein-Friesian Cattle.

LESTER O. GILMORE, WM. E. PETERSEN AND J. B. FITCH, University of Minnesota.

Increased knowledge of color inheritance in cattle is associated with a revival of interest in attempts to associate color genes with those responsible for function, structure and with the further attempt to verify authenticity of registration. Beginning the study at Kansas, Ibsen has pointed out the distinction in the conditions occurring in Holstein and Herefords, although the appearance of the head in the two cases is similar. In 1933, Minnesota joined in the study, the results of which are here summarized. The details for both the Kansas and Minnesota findings are planned to be published concurrently.

Positive identification of this pattern is sometimes difficult because of the lack of pigmentation on the body, a condition thought to be related to decreased pigmentation on the head. Thus a "white headed" animal that is largely white may have small pigmented areas close to the eyes because of the presence of complicating genes for distal black are epistatic to dominant whites in general. Another difficulty in identification comes from the presence of the same genes for black on animals otherwise containing but little white. This latter combination may result in animals appearing somewhat as though they had a blaze.

The "white head" pattern was found to be present on 8.6 per cent of 94 animals in a herd containing transmitters of the responsible gene as compared to a 5 per cent frequency in a population of 3655 animals included in a group selected for comparison. This group included all animals whose picture appeared in the Holstein-Friesian World in all issues from 1910–18 and 1924–25.

The results indicate that the condition for the "white head" pattern is dominant or epistatic to other head patterns that are uncomplicated by genes affecting color on distal areas. This conclusion results from a consideration of both parents of 31 "white head" individuals as well as pictorial pedigrees of "white heads." It was found that "white head" individuals have at least one "white head" parent and that for some individuals the presence of the pattern can be traced in this manner through at least nine generations.

Most of the "white heads" studied were traced back to Hengerveld De Kol or to a closely related ancestor. The possibility exists that if all "white heads'' studied had been traced further they, too, would have been found to trace in direct line to a common ancestor.

P36. The Inheritance of Fertility in Dairy Cattle. ARLESS SPIELMAN AND I. R. JONES, Oregon State College.

Animal breeders have long been of the opinion that families and breeds of livestock vary greatly in their inherent capacity for reproduction. The belief that these differences are due to the presence and expression of genetic factors is indicated by a review of the literature dealing with fertility in Drosophila and other species.

A study was undertaken to determine if genetic factors affecting fertility have been operating in the dairy herd of 368 cows maintained by the Oregon State Agricultural College during the past 25 years.

Fertility in the dairy cow obviously depends on two factors, the frequency of reproduction, and the total number of successful gestations.

The per cent reproductive efficiency has been proposed in an earlier study by the authors, as a measure of the frequency of reproduction. In the study reported, a significant difference was found in the mean per cent reproductive efficiency among cow groups and between breeds.

A correlation of $r = +.546 \pm .118$ between the reproductive efficiency of the foundation cows and the mean reproductive efficiency of their respective female descendants was obtained.

A ''longevity-of-fertility'' rating is proposed as an expression of the number of successful gestations which a dairy cow undergoes. A significant difference in the longevity of fertility among the four breeds studied was observed. The values were as follows: breed No. 1, 32.56 ± 3.19; breed No. 2, 36.47 ± 5.53; breed No. 3, 63.52 ± 7.35; breed No. 4, 25.54 ± 4.37.

Correlating the per cent reproductive efficiency with the ''longevity-of-fertility'' rating, a significant value of $r = +.804 \pm .026$ was found. This close relationship suggests that the reproductive efficiency of a cow may be a good indication of the probable number of successful gestations which she may undergo.

Although a comparatively small number of animals were included in the study, it is believed that the high correlation values obtained indicate that inheritance is in part responsible for the great differences in fertility of dairy cows.

P37. Analyses of Dairy Herd Breeding Records. S. W. MEAD AND W. M. REGAN, University of California.

A diagrammatic method of presenting the history of reproduction in dairy herds, reported in the JOURNAL OF DAIRY SCIENCE for June, 1938, has been used in a study of the efficiency of reproduction in seven of the dairy herds cooperating with the University in its Jersey breeding experiment.

Complete breeding and calving records have been maintained continuously over periods of from six to ten years, during which time these herds have averaged 48 females of breeding age. All herds have been free of both Bang's disease and tuberculosis.

The analyses of the data have included, (1) a study of the average number of services required per pregnancy in each herd during each succeeding two year period, (2) the average number of services required for first pregnancy compared to all later pregnancies and, (3) the number of services required for first pregnancy, among heifers requiring three or more services, as compared to the number of services required by the same heifers for later pregnancies.

To obtain the average number of services for pregnancy during a two year period, the total number of services recorded during the period were divided by the total pregnancies occurring during the same period as determined by later recorded calvings. According to the method used in this study, four of the seven herds have had a reasonably good reproductive history over a period averaging eight years. These herds have averaged less than two services per pregnancy. The other three averaged 2.6 services. Each of these last three herds has experienced more difficulty with heifers being bred for first pregnancy than have the other four. In one herd over a period of eight years 57 per cent of the heifers have required three or more services for first pregnancy. Contrary to common opinion, all herds do not experience more breeding trouble with heifers being bred for first pregnancy than for later pregnancies. In only two of the seven herds have the heifers averaged more services for first pregnancy than for cows bred for later pregnancies. In all herds, regardless of the extent of breeding difficulty among heifers, our figures have shown that the heifers requiring three or more services for first pregnancy, required fewer services for all later pregnancies. At no time during the histories of the two herds experiencing most trouble with heifers being bred for first pregnancy did later pregnancies require as many services as first pregnancies.

P38. Increasing Production in Institutional Herds. A. L. Beam, C. S. McLearen, and A. A. Borland, The Pennsylvania State College.

Because there has been a substantial increase in the production of milk and butterfat in a number of state institutional herds during the last few years a survey of fourteen of these herds was made to determine the causes and the variation of this increase among the herds. It was hoped that through this study additional information could be obtained which might aid in maintaining or improving production in dairy cattle.

Data was gathered from each institution by a series of arranged visits at which times information was secured by questioning the herdsmen, by studying the records available, and by inspecting the herds and facilities for dairying at each institution.

About 1,200 Holstein cows were included in these institutional herds at the time the survey was made. The size of these herds varied from 21 to 145 milking cows. All herds are entered in Dairy Herd Improvement Associations and uniform management records covering milk production, feed consumed, and breeding data are kept by each herdsman or farm manager. With the exception of a few years in several herds these records have been kept since each herd was established. A study of the records show that the production of milk for the herds as a whole has been increased from a little over 9,000 pounds of milk in 1929 to over 12,000 pounds in 1935. Since 1935 the average yearly production for all herds has not been less than 12,000 pounds of milk. During the last six years the average production among these fourteen institutional herds has ranged from 9,720 to 16,667 pounds of milk.

The preliminary findings indicate that the improvement has been too rapid to be attributed solely to breeding and selection. Evidence seems to point to the conclusion that management factors have had a marked influence on the increase in production. Careful culling, shortening the length of calving interval, disease eradication, and good management practices have had the major influences in increasing production for the herds as a whole.

Age of first calving, amount of culling, type of foundation animals, demand for milk by the institutions and prevalence of diseases are major factors affecting the variation of production between herds.

Disease eradication relative to breeding troubles has increased the average production of all herds. In 1932, 18 per cent of the cows in all herds required three services or more per conception. In 1937, only 12 per cent of the total number of cows required three or more services per conception. The herds having a lower average yearly production have had repeated outbreaks of Bang's disease and mastitis. The herd maintaining the highest yearly production has been free from Bang's disease and has had practically no trouble with mastitis.

The age of first calving at the institution having the highest yearly production over a period of years has been 32.5 months. Growing heifers out well before breeding has been practiced in this particular herd for the past twelve years. At several institutions with much lower yearly production it has been customary to have all heifers freshen at an average age of 27 months.

One very definite fact was brought out relative to the transmitting ability of related sires. In one institution fifteen daughters of one sire averaged 11,808 pounds of milk while their dams had an average production of 13,787 pounds. Seven daughters of this same sire in a private herd averaged 10,870 pounds of milk as compared with 19,566 pounds for the dams. All records have been converted to a mature basis.

In another herd a close relative of the above bull sired fifteen daughters whose average production was 21,239 pounds of milk. The dams of these daughters had an average production of 16,892 pounds.

This is further evidence that a bull's pedigree is not a safe measure of his transmitting ability. In large herds it would pay to prove a young sire before using him extensively.

P39. The Use of Various Oils and Fats for Calf Feeding. T. W. Gullickson and F. C. Fountaine, University of Minnesota.

The original object of the experiment was to determine whether some cheaper oil or fat can be used satisfactorily to replace butterfat in the diet of calves. The following were used. 1) soy bean oil, 2) corn oil (Mazola), 3) cottonseed oil (Wesson oil), 4) lard (Silver Leaf brand), 5) butter oil.

The experimental milks as fed were prepared by homogenizing each oil or fat into fresh skimmilk at 3,000 pounds pressure, single phase, to form a product containing 3 5 per cent fat.

Two to four calves were used in testing each skimmilk-oil product. For purposes of comparison, one calf was fed normal whole milk, and another, a ration designed to be almost fat-free.

The calves used were grades of Holstein and Guernsey extractions, males and females, and healthy and thrifty when placed on experiment. They were fed whole milk until about one week old, after which, excepting for the two calves mentioned above, the skimmilk-oil product was gradually introduced. From 20 to 30 cc cod liver oil (U.S.P.) was fed daily. Beginning at about 30 days of age a limited amount of a low-fat concentrates mixture was added to the diet. The calf on the fat-poor diet was fed a mixture of 2 parts dry skimmilk and 1 part glucose (Cerelose). All calves, except those fed lard, were fed on the same plane of nutrition. The latter were fed at slightly higher level.

Each calf was kept in a separate pen, fed individually, and weighed weekly. Blood fat was determined weekly.

Results. The calves consumed the diet prescribed. Indigestion appeared; the soy bean oil group being most seriously affected. Two of three calves in this group died by the 13th week. The third, weak and unable to stand by the 17th week, made remarkable recovery when fed whole milk. Average weekly gain per calf was 2.3 pounds.

One calf fed corn oil died within 10 weeks, gaining total of 15 pounds. Weekly gain of other two for 18 weeks about 3.25 pounds. Of the three calves fed cottonseed oil, one died the 12th week, total gain 13 pounds. Weekly gain of the others in 18 weeks, 2.75 pounds.

No differences were noted in appearance between calves fed whole milk and those given the homogenized skimmilk-butter-oil product. Average weekly gain of both groups, 18 weeks was about 8.5 pounds. The low-fat diet calf was rough but gained nearly 5.5 pounds weekly. Lard-fat calves remained thrifty throughout. Average weekly gain about 5 pounds.

Many of the vegetable-oil-fed calves developed a dermatitis-like condition resembling that described by Burr occurring in rats on a diet low in linoleic acid. Blood fat varied widely but averaged lower in the low-fat and the soy bean oil calves and higher in the cottonseed oil and corn oil groups than in the calves receiving butterfat. Post mortems revealed no marked abnormalities except an almost complete absence of internal fat.

P40. The Influence of Fineness of Grinding on the Digestion Coefficients of Oats for Dairy Cows. G. C. WALLIS, T. M. OLSON, AND T. A. EVANS, So. Dak. Agric. Exp. Station.

The effect of the fineness of grinding on the digestion coefficients of corn have been previously discussed. A similar set of observations have been made on oats as it seemed quite possible that the results with grains protected by a heavy seed coat, or hull, might be different from those obtained from other grains, such as corn. Four cows in mid-lactation were used on these trials with oats. The investigation consisted of a series of four digestion trials using the four cows on each trial. Each trial consisted of a preliminary period of 7 days and a collection period of 14 days. The rations were adjusted to meet the average requirements of the cows for the entire experimental period of approximately 3 months, so there was no change in the quantity of any feed being used throughout the duration of the experiment. All the rations consisted of half by weight of alfalfa hay and half by weight of oats. The amount fed was adjusted to the average requirements for each cow and thereafter the only change was in the preparation of the oats. In the first trial medium ground oats were used; in the second, finely ground; in the third, whole oats; and in the fourth, alfalfa hay alone. In the third trial the feces were washed over a fine screen and the whole oats which had passed through the digestive tract were recovered. The amount of oats recovered in the feces varied from 11.57 to 19.91 per cent for the different cows. All the chemical analyses for this experiment have now been completed and the final results are being calculated at the present time. When these are available interesting comparisons between the results on corn and those on oats will be possible.

P41. The Effect of Changes in Environmental Temperature on Water Consumption of Producing Dairy Cows. W. M. REAGAN AND S. W. MEAD, University of California.

The effect of changes in environmental temperature on high producing dairy cows was studied in a large psychrometric room in which the temperature was increased from 40 to 100° F., while air movement and relative humidity were maintained at constant values of 50 feet per minute and 60 per cent, respectively. Data were taken on three pairs of Holstein cows for periods approximating three months. A standard diet of hay and concentrates was fed in accordance with individual production and appetite. Each cow was provided with an individual water bowl to which water was supplied through a carefully calibrated meter. Daily recordings were made of the feed and water consumption and milk production of each cow.

A study of these data fails to substantiate the general belief that the water consumption of dairy cows is materially increased in hot weather. Uniformly constant values were obtained for temperatures ranging from 40 to 80° F. The upper limit of heat regulation for producing Holstein cows is about 80° F. When the room temperature is maintained above this point for more than 24 hours, the animals tend to become hyperthermic, coincident with a reduction of feed and water intake and the milk "flow." Instead of an increase there is an actual decrease in the amount of water drunk at the higher temperatures.

It has long been recognized that there is a definite relationship between the cow's water requirement and her food consumption and milk production. When, in the study of our data, proper correction was made for the decline in food intake and milk production, there was only a slight correlation between room temperature and water consumption.

EXTENSION SECTION

E1. Community Dairy Cattle Breeding Plans. Report of Sire Committee, J. F. Kendrick, *Chairman*, U. S. Bureau of Dairy Industry.

The general objective of all organized dairy cattle breeding programs is to improve dairy herds by making available at reasonable cost, continuous superior sire service for the herds of the greatest possible number of dairymen. Various types of organized dairy cattle breeding programs are at present being conducted in the United States. The cooperative bull association is one type of organization that is proving most successful when it is carefully organized and operated on a sound business basis. In recent years other types of organizations have been set in operation in the various States, all designed to fit the particular conditions under which they operate. The Sire Committee shall attempt to make a complete report on the various types of organizations now in existence, pointing out the apparent merits and weaknesses of each.

E2. Bull Studs in the Community Dairy Cattle Breeding Program. Ivan H. Loughary, University of Idaho.

A community effort to obtain low cost, high quality bull service by herd owners agreeing to patronize a selected individual who purchases suitable bulls is a new program in Idaho. The group of bulls is called a bull stud.

It may be composed of bulls of several breeds. Bull service is sold delivered to the dairyman's farm. A patronage agreement is signed by both the bull stud operator and the dairy herd owner.

The plan is not a substitute for Cooperative Bull Associations, but rather supplements them by being more satisfactorily operated among small herds.

Seventy per cent of the cows of the country are in herds of 10 or less, and the average number of cows per herd is less than 6. It is evident that the field for bull studs far exceeds that of herds of suitable size for successful operation of bull associations.

In Idaho bull studs, one bull is mated with three or four times as many cows each year, as are bulls in Idaho Cooperative Bull Associations.

In nine Idaho bull studs are 36 bulls which are breeding annually more than 4000 cows. These Bull Studs are supervised by a committee which meets once each month to audit the records of operation, pass on bull purchases and adjust unsatisfactory management factors.

Higher breeding efficiency is experienced than in Cooperative Bull Associations, possibly due to a douche being administered to the bull immediately following each service. Only one good service per cow and two cows per day are bred by Stud Bulls. Fees range from $3.00 to $4.00 for the initial service per cow, and $1.00 for each return service. Conceptions on the first service averaged 68 per cent during 1937 and about 80 per cent during 1938.

Careful feeding is given the bulls to maintain them in good physical condition and avoid too large barrel or over or under condition.

The plan is more satisfactory to the small herd owner than is a Cooperative Bull Association. The individual herd owner has no investment, is at a minimum of inconvenience, obtains high quality bull service at a cost below that of owning even a scrub bull, no disease spread has been experienced, few complaints manifest, and a constructive breeding program is established.

E3. Extension-Farm Security Bull Associations in Utah. LYMAN H. RICH, Utah State College.

Utah farms are small. More than 30,700 families live on 1,324,000 acres which allows about 43 acres of irrigated land per farm. Twenty-eight per cent of the farms are under 20 acres in size.

The Farm Security Administration has 4,200 individual loans and 4,000 cooperative loans. At least 25 per cent of the farms have a very low income level as indicated by these loans. To meet expenses, unit yields and high unit production is paramount on these farms. Seventy-eight per cent of the farms in Utah have dairy cows, averaging about five in number. Too many inferior bulls have been maintained for small herds. Reducing the number and improving the quality will raise the much needed farm income.

Cooperative bull blocks and associations have rapidly increased in Utah the past two years to meet this problem of reduced expenses, and marked improvement in quality of herd sires has been made. These bull blocks are supervised cooperatively by the Extension Service and Farm Security Administration. A total of 61 such units have been organized, involving 370 owners and 343 users who sign patronage contracts. These 713 farmers own 3,535 cows cooperating in the plan. Utah farmers live in central towns and villages so that short distances make bull blocks less expensive than other systems. Forty-one of these cooperative blocks have been organized into 13 associations of 3 or 4 blocks each.

These units have their beginning through the need of a loan obtained by a group of farmers through the local Farm Security supervisor, the funds coming from the Farm Security Administration at 3 per cent interest, payable on a five-year plan. These farmers usually have a separate farm loan with the Farm Security and therefore are closely supervised financially. When several blocks in a given area are completely set up, an association is organized. The Extension Service and Farm Security representatives meet all dairymen in these respective groups and consider the constitution and by-laws which have been carefully drafted. Discussion follows relative to quality of herd sires, rotation plan, replacements, insurance, disease control, and other details. Representatives of each block make up the association board, usually five in number. Organization of associations, selection of sires and educational activities are supervised by the Extension Service. The Farm Security assists in organization work and supervises the loans.

Bulls are insured for $100 each through a private company at $7 per year. A fund of $10 per block is set aside for replacement also. Purebred breeders cooperate in furnishing sires with production records at a nominal cost.

Bulls are maintained in suitable pens, 60 feet by 20 feet, minimum size with an open shed dry and free from draft. Service fees range from $1.50 to $2, the number of cows ranging from 40 to 100.

Testing for Bang's disease is a requirement. The loans are paid out in five years' time. This plan has been in operation two years only.

E4. Progress and Prospects of Organized Artificial Insemination of Dairy Cattle. E. J. Perry, New Jersey College of Agriculture.

According to a questionnaire returned by most of the extension dairymen, 17 cooperative artificial breeding associations with an average of 76 members, 855 cows and 5 bulls were operating in ten states on April 15th, when this was written. The states are: Connecticut, Maine, Missouri, Minnesota, New Jersey, New York, Ohio, Pennsylvania, Tennessee and Wisconsin. Other states reporting associations being formed included California, New Hampshire, Montana, Rhode Island, Vermont and Washington. Interest was reported as developing in several others.

The extension men are practically unanimous in the opinion that cooperative artificial insemination possesses exceptional advantages in dairy cattle breeding. There is general agreement that the three leading ones are these: (1) It makes possible the wide use of outstanding sires, (2) the sires can be proved at a younger age and more effectively than is normally the case, and (3) it also reduces the disease hazard. The principles of genetics can be applied on a wide and practical scale. The survey also revealed an almost unanimous sentiment in favor of veterinarians doing the work for an association. The relation of disease to breeding efficiency renders the services of the veterinarian one of the outstanding features of an artificial breeding program. There are four conditions which need increased attention in dairy herds today. They are: (1) spreading of disease by the bull passing it from one cow to another, (2) diseased semen of the bull, (3) diseased genital organs of the cow and (4) the check-up examination for pregnancy. The losses from any one of these are cutting deep inroads into the profits of the dairymen. These losses can be greatly reduced by a well conducted program of artificial insemination.

The number of inseminations required for those cows becoming pregnant is less than 2 with an approximate average somewhere between 1.7 and 1.8. One association reported 1.3. It is assumed that its herds are predominately Bang's disease free. The yearly chargę per cow, covering three inseminations, ranges from $3.00 to $6.00 after the paying of a herd entry fee of $5.00 or $10.00. The bulls are from one to twelve years of age and the difference in fertility even among young ones is reported as being marked.

In most of the associations the members expect a very high rate of conception. This is unfortunate and due to the wide publicity given the project by the press and to the fact that very few know what their conception rate was by natural breeding. A lot of available data indicate that the rate for both methods is running practically the same. It is, therefore, necessary to convince present and prospective members that the new system is not a panacea for the many ills that may befall a herd. Those who have now had experience with the project wish to caution that sires must not be overworked and that at least three and sometimes four are necessary when semen must be taken every day. Plenty of exercise is urged as an aid in keeping the bulls in good condition. Time and labor can be saved in keeping them at a central point regardless of how much the association expands. An association should be organized with care, and only after a healthy demand has come from the dairymen themselves and when outstanding sires and a well trained veterinarian are available.

E5. Utilization of Proved-Sire Records and Herd-Analysis Data. Report of Sire Committee, J. F. KENDRICK, U. S. Bureau of Dairy Industry.

The necessity for the complete utilization of data obtained in the dairy herd-improvement association identification and permanent record program is obvious, if the general program of dairy cattle improvement is to achieve its greatest success. Different methods of utilizing proved-sire and herd-analysis tabulations are used in different states. The Sire Committee shall attempt to bring together all the various methods used and offer recommendations as to those methods which appear to have greatest merit.

In addition to the report on the utilization of DHIA data, the committee shall attempt to bring together in a report those practices followed in the various states to make the Dairy Herd-Improvement Association Identification and Permanent Record Program most effective in the field. Particular attention will be given the various methods employed to encourage the association testers to keep the record work and reports up-to-date.

E6. Utilization on State and County Basis.* WARREN GIFFORD, University of Missouri.

The extensive use of representative herd and sire analyses, presented in chart form, have been used as a demonstration method of teaching the fundamentals of dairy cattle breeding to breeders in county and state meetings.

These analyses have been particularly helpful in dairy cattle breeding schools to illustrate how breeders have used the four systems of mating in successful herd and community breeding plans. Three distinct types of herds have been used, (a) herds periodically outcrossed with beef bulls, (b) high producing herds developed from mediocre foundation grade stock, (c) purebred herds developed by a well planned breeding program. The results obtained give a clear understanding to the underlying principles and technique in the proper evaluation of production records with reference to measuring genetic and environmental factors. They emphasize the value of brood cows and proved sires, the necessity of uniformity in feeding and management practices, the value of a disease control program, continuous testing and permanent identification of all cattle. The material also provides a basis for group discussion on developing breeding plans, available sources of seed stock, data necessary to evaluate breeding animals efficiently and the many other questions involved in the individual and organized community breeding program.

Herd analyses have also been used advantageously in farm breeding plan demonstrations, breed association tours, dairy herd-improvement association annual meetings, dairy herd-improvement association membership drives and the county bull book and sire exchange program meetings.

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* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 607.

E8. Influence of Disease in Dairy Cattle Breeding Programs. GEORGE E. TAYLOR, New Jersey College of Agriculture.

Disease control problems are of major importance to the breeder of dairy cattle. Failure to control and prevent disease may quickly erase the influence of a lifetime or generations of constructive breeding. The value of an animal for either breeding purposes or economical production is first of all dependent upon the animals' health status. Realization that animals cannot demonstrate their value as desirable breeders or transmitters before 5 years of age or older, emphasizes the great importance of disease control. The control of infectious disease becomes more difficult with advancing age. Attention should be focused on the importance of disease prevention rather than disease treatment.

The service and influence of proven sires is being extended through the sale and exchange of bulls, Cooperative Bull Associations, Community Bull Studs and organized artificial insemination units. Diseases of major importance in bulls, especially proven sires, regardless of program being followed include T. B., Johne's, Bang's, Trichomoniasis and Sterility. In the case of proven dams, mastitis should also be included. Calf scours, colds and pneumonia are the principal causes of death and subsequent loss of valuable blood lines in young animals. Anthrax, Blackleg and Hemorrhagic Septicemia may present a local problem in some sections in addition to other minor ailments.

The importance of herd health and recommended control methods and practices is first of all a problem of education on the part of the breeder. An educational program should include and emphasize the following important points:

1. Relationship between proper feeding and herd health including liberal use of quality roughage.

2. Relationship between management practice and herd health including home grown replacements, size of unit, adequate housing facilities and accurate complete health and breeding records.

3. Complete information concerning specific diseases including: cause, etiology, symptoms, immunity and practical control and preventative measures.

The organization of an integrated and coordinated educational disease control program should include the following agencies cooperating: 1. Extension Service; 2. Experiment Station; 3. State Bureau of Animal Industry; 4. State Veterinary Medical Association; 5. U. S. Department of Agriculture.

The Extension Service should logically take the lead in an educational program including meetings, demonstrations, distribution of bulletins, circulars and mimeographed material with the State Bureau of Animal Industry and the State Veterinary Medical Association cooperating. The function of the Experiment Station and the U. S. Department of Agriculture is fundamentally research concerning disease. The State Bureau of Animal Industry and State Veterinary Medical Association cooperating are responsible for all state and federal health regulations and accreditation of clean disease-free herds. Closer cooperation of the above existing agencies is highly desirable.

E9. Should We Unify the Extension Program? C. L. BLACKMAN, Ohio State University.

Some extension workers have a growing conviction that the agricultural education program should be built more largely around the solution of major farm problems. They place emphasis on the fact that the farm business as a whole is more important than any single enterprise in it. They believe that the solution of many of these problems requires the contribution of more than one department. If this is true it seems to follow that each department involved in the solution of a problem should consult with other departments in order to present a better correlated procedure.

Although it can hardly be said that the different subject-matter departments now plan their programs entirely without regard to the other departments, the question arises, "Is sufficient effort made to relate one subject to another? If not, does the lack of an integrated program of extension education result in each department being less effective than it could be?"

Perhaps the point can best be illustrated by an attempt to meet a serious farm problem in Ohio. According to the records, wheat production during the past 60 years has increased about three bushels per acre, and stands now at 18 bushels. Corn production has remained static at about 37 bushels per acre. The Agronomists point out that improvement in seed and methods of production should have caused a 40 to 60 per cent increase in the per acre production of these crops.

Agronomic investigations indicate that depletion of soil fertility and excessive erosion are chiefly due to unscientific methods of tillage and the growing of too many acres of soil depleting crops such as row crops and small grains and not enough acres of rotation hay and pasture, which are soil building.

Farmers endeavoring to bring their farms into productivity balance found that they were not only faced with the agronomic problems of rearranging their rotation, but as a result of these changes, were faced with the possibility of seriously revising their whole farm program. Dairy farmers were wondering if it was necessary to increase their herds in order to consume the extra hay and pasture which might be produced as a result of cropping changes. At least three departments might make some contribution toward the solution of this problem. (Agronomy, Rural Economics and Dairy Husbandry.)

In the fall of 1937 after conferences with Agents and Specialists in these three departments, it was decided that a series of meetings would be held dealing with each phase of the problem. Each specialist endeavored not only to discuss his particular angle of the problem but also tried to relate his subject-matter to that of the others. The interest in the first series of meetings was so satisfactory that the plan was expanded in 1938 to include the Department of Agricultural Engineering. The meetings were arranged at conferences of agents and specialists. The specialists also met and discussed their different plans of approach and then each specialist prepared an outline of his discussion to be handed to farmers at the time of the meeting. A special effort was made to relate each type of subject-matter material to the others.

This plan is not suggested as the only method of carrying on extension work. Results indicate that it has merit and that its use will help specialists and agents to build a more unified program. The combination and number of departments involved will depend on the natures of the problem.

E10. Michigan Dairy Planning Activities. A. C. BALTZER, Michigan State College.

Endeavoring to develop a long time dairy, project in a county with the cooperation of local farmers and dairymen, creamery and dairy processing organizations, educational forces and others will assist the college specialist and the county agricultural agent in establishing a more permanent and a more progressive county and state dairy program.

First of all the local people can choose thoughtfully and wisely if they are given by the specialist the national, the state and the local facts and factors which influence for good or evil their dairy farm business.

For ten years in Michigan such action programs have been emphasized and they have been helpful and fruitful. The local people, dairy farmers and others gain a better vision of the work and the purpose of the college specialist and the county agricultural agent. Our purpose is to develop, create and establish on the majority of Michigan dairy farms a more balanced type of dairy farm operation which will develop permanency and lend improvement to the standard of farm living.

How do we do it? The following projects have been used in Michigan as a means of focusing attention on our aims and we strive to select just one or two activities each year as a feature in the local program. The county dairy planning project serves to head up all the dairy activities in a county when it is practical to use it. From this project a dairy record testing program is offered which includes the D.H.I.A., H.I.R., and A.R. activity. Closely associated with these production testing programs is the Better Sires project which includes bull surveys, bull clubs and associations, baby bull calf placements, artificial insemination projects, bull pens, college bull lists, conducting breeding schools, district and county bull display days, sire pledging, use of sire proofs, and herd analysis programs. The fourth project is dairy feeding and dairy pasture improvement and management which is encouraged through use of winter schools, winter and summer field and barn tours, creamery and condensery haulers, and elevator men schools, D.H.I.A. summer and winter events, etc. Activities for quality milk and cream are manifold and depend upon the local planning committee's wishes and economic influences. They are closely related with a needful dairy utilization program among farm producers. Cooperation with the state regulatory forces is valuable to explain the sanitary milk and cream laws before county wide meetings. Master butter makers contest, creamery check-up, milk and cream scoring in high schools, annual meetings of creameries, cooperating with district station men and with cream and milk haulers serves to create action by getting processing agencies to install cooling tank service, whitewash service, and assist in distributing dairy products back among producers. Farmers will eat dairy products in great volume and of all kinds if the processing agencies will service the producers. Demonstrations before women home economics groups, P.T.A., etc., are useful, and cooperation with county school commissioners, county medical units, dairy and foods councils in large urban centers are extremely desirable.

It is necessary that the dairy specialist carry through the activity selected, work with the local committee, and next year review the program, report the successes and failures, and counsel with the local leaders further for the ensuing year's effort.

These and other projects which are supplementary when explained before a committee of farmers, dairymen, D.H.I.A. members, processors, urban and rural leaders, will result in their selecting some activity which can be wholesomely conducted in the year to the good of the dairy buisness, to the effective furtherance of the county agents dairy program and add dignity and respect to the state college and the dairy specialist.

E12. Annual Dairy Herd Improvement Association State Reports or Summaries; Their Preparation and Use. ROGER W. MORSE, Oregon State College.

The first dairy herd improvement association was organized in Michigan in 1906. At the present time associations are operating in each of the 48 states and in the islands of Porto Rico and Hawaii. There were 1,228 associations in operation January 1 of this year.

These associations are business organizations of farmers who are organ-

ized into an association and a man hired to keep cost accounts on their cows. The work of dairy herd improvement associations is supervised by the County and State Extension forces and the United States Department of Agriculture.

Since the work was started, in most states there has been an annual audit, report or summary made of the year's work and results by each association. For a good many years, most states have taken these association reports and other information assembled during the year and compiled them into an annual state report and summary of dairy herd improvement association work. These state summaries have been used to give the members, testers, and county agricultural agents in the counties a measure of the scope and results of testing in their counties as compared to other counties of the state. They have been useful as a measure for State Extension dairymen to use in determining progress of their projects.

As this work has developed and grown, the use of testing records has expanded from merely being a basis for culling and feeding, to include compilation of data on breeding practices and on the locating of sires and cows that transmit desirable production and type characteristics to their offspring.

This paper presents a brief outline of the kind of state dairy herd improvement association reports or summaries prepared by the different states. They vary from a single page tabulation of association average production and feed costs to rather elaborate mimeographed or sometimes printed reports and analyses on testing records.

There is a wide variation on the type of information assembled and the uses made of these reports The relationship of the size of the dairy industry in the different states to the number of Extension dairymen employed, the number of dairy herd improvement associations, and the type and use made of the summaries is discussed.

E13. D. H. I. A. Methods and Results in California. G. E. GORDON, University of California.

Dairy improvement, through cow testing association work, has been carried on in California since 1909. It was not until 1921, however, that the dairy improvement program was organized on a state-wide basis. A study of the situation at that time based upon the 1920 census indicated that average production per cow was not high enough to develop a stabilized and profitable dairy industry. It was found that the industry was of sufficient value to the state to justify efforts to put the industry on a permanent and profitable basis.

In order to do this it seemed desirable to increase the average production per cow of all cows in the state. A state-wide program was, therefore, adopted on a basis whereby a relatively high percentage of dairymen of the state would participate. This program was designed to aid in increasing average production through culling of low producing cows and through better breeding and feeding practices. All of these improvement practices were, of course, to be based upon production records through rather widely developed testing associations. These associations were to be organized on a basis which would provide sufficient income to keep competent testers interested in the work and still provide the service at reasonable cost to the dairyman. This program was, therefore, based on associations consisting of rather large numbers of cows per unit. This system also eliminated the possibility of testers keeping detailed feed records on the individual cows in members' herds. Furthermore, it seemed desirable that dairymen be encouraged to accept the responsibility for carrying on the detailed feeding program rather than depend upon the testers to do it for them.

In the early years of the program the associations were organized on a yearly basis. This system had certain inherent disadvantages. A change in policy was therefore developed to encourage a longtime point of view toward testing. This policy minimizes the cow testing year as such and emphasizes continued service to members rather than placing emphasis on the securing of new members. Under this plan dairymen are not signed on contract for any specific period of time, but are free to withdraw at any time they desire. The associations accept the responsibility for providing such service in the use of records that dairymen will see the advantages of continued testing.

Experience in our program indicates that success depends upon definite objectives with an organized program to achieve such goals. Any program to achieve widespread results must be widespread in its application. Members of associations must accept certain responsibilities in carrying out the program, rather than having the tester assume those responsibilities for them. Contacts must be maintained with members to insure continued and adequate use of records.

E15. Analysis and Use of D. H. I. A. Feed Records. C. G. CUSHMAN, Clemson Agricultural College.

The more definite and exact evidence agricultural workers can gather which represents the teaching of research data applied to all sorts of differing farm conditions, the more they can divorce themselves from opinionated ideas and the more effective they will be. Of equal importance is putting these facts into visual shape so that they can be easily seen.

There is latent in the great mass of D. H. I. A. feeding records, facts of unusual character which can be turned into powerful working tools that will set dairy specialists upon the solid rock of "facts" as he deals with varying and sometimes puzzling sets of circumstances.

The feed cost of milk production represents from 60 to 70 per cent of the total cost. The dairy farmer may quickly influence this 60 or 70 per cent of his total cost by herd and farm management.

The most elementary analysis of feed records is the grouping of herds according to practice based on information in testers monthly reports. Such grouping in South Carolina in February 1938 on the basis of annual grazing versus no grazing showed $4.03 per cow greater return over feed cost and 45 cents per 100 pounds saving in feed cost in favor of annual grazing. In February, 1937, two herds are compared in the months of December, January, and February, one having adequate annual grazing and the other insufficient annual grazing, other conditions being similar, to show a striking difference in favor of abundant annual grazing.

Heavy grain feeding *versus* moderate grain feeding according to the October 1937 monthly report showed a gradation downward trend from a feed cost of $1.80 per 100 pounds to $1.08 per 100 pounds.

An analysis of June through November reports on one herd show 33.2 tons of hay equivalent grazed from 12 acres of kudzu by 35 cows.

The December 1938 monthly report showed substantial evidence of lowered cost and higher milk yield in favor of herds fed on a program of all silage and no hay with grain as compared to herds feeding silage plus other roughage and grain.

Herd analysis charts are prepared showing a year's picture by months of the behavior of herds as to feed requirements, feed fed, average milk per milking cow, average milk all cows (including dry), and feed cost per 100 pounds milk. Statistical and chart analyses on two herds are shown.

All cows on test in 1937 are grouped according to type of market and a study made of the source of feed nutrients. Analysis shows that high or low prevailing price of dairy products indicates a feed production program which should furnish nutrients in either the cheaper or the more costly feed sources. This analysis is set forth in several tables which correlate sources of feed nutrients with price of milk in different types of market.

E16. Progress of Demonstrations with Grass and Legume Silage.

DWIGHT M. SEATH AND JAMES W. LINN, Kansas State College.

Field reports indicate a growing interest in acid and molasses-treated silages. These special treatments which permit the preservation as silage of the legume and grass crops are now being adopted by widely-scattered dairymen even though they are hardly out of the experimental stage. Field workers in dairying need more information pertaining to the practicability of such treatments as well as the best methods to advocate in their use. They also need to know the relative merits of the methods as well as conditions under which each method should be recommended. Comparative results based on demonstrations within the various states will do much to supplement information gained from experimental work as a guide for future recommendations.

There are many reasons advanced for the growing interest in grass and legume silage. For certain dairymen, this method of feed preservation offers a solution for the better utilization of the first crop of legume hay which otherwise is often damaged by rain. Some have the same object in mind but use the cereal grains, such as oats, for the silage crop. Others provide mid-summer pasture from the silo by the preservation of a high-protein legume or cereal crop in the spring and feeding it in July and August, thus maintaining production and keeping up the quality of the milk produced. Still others use the acid or molasses method of preservation solely as a means of fortifying their milk with carotene, thus permitting the production of spring-colored milk throughout the year. A combination of these reasons plus others not mentioned motivate many to use these special silages.

Practical objections to these special silages include the cost of the various preservations, the labor and inconvenience of treating silage materials, the variation in the palatability of the silage depending upon the maturity of the crop or the amount of preservative used, and the labor and lack of machinery for conveniently handling the green forage material. With more experience, dairymen will devise and standardize methods which will eliminate many of the existing objections.

The results of a survey made of Kansas dairymen who have been using either the acid or the molasses method of treating silage crops will be a part of the complete report given on this subject.

E17. Pasture Improvement as a Project in the State Dairy Extension Program. O. J. Hill, State College of Washington.

Pasture improvement has been carried out as a cooperative project with the Soils and Crops specialist. Due to a great diversity of conditions no one program is adaptable to all parts of the state. In order to develop interest in grasses best adapted to different regions, rod row plantings have been used extensively.

Permanent plantings are encouraged wherever feasible. Cooperators are selected to seed approved mixtures according to best agronomic practice. Tours are conducted to these demonstration plantings during the grazing season. Some demonstration plantings have been in for 18 years.

Carrying capacity studies on dairy farms have been made; however measurable results are difficult to secure due to varying stands of grass and methods available for collecting data. Permanent pastures on good bottom land at the Western Washington Experiment Station have produced 5498 pounds of digestible nutrients per acre. This is more nutrient than can be grown with other crops in this area. Carrying capacity has been increased to 5985 pounds following rotational grazing.

Farms that have developed adequate permanent pasture to meet their requirements during the dry season usually have a surplus of forage during April and May. Certain plots are not grazed at this time and are cut at an early stage of growth for silage. This silage has been preserved either in stacks or in tower silos.

Pasture fertilization has been a definite part of this program. In order to emphasize the importance of manure in pasture fertilization, some demonstrations on good manure storage systems are used each year. Special emphasis has been placed on the use of liquid manure. Phosphate fertilizers are valuable in most cases to supplement either liquid or solid manure.

Other management practices emphasized are: harrowing, clipping, reseeding, and danger of overgrazing.

E18. Report of Quality Improvement Committee. C. J. BABCOCK, *Chairman,* U. S. Bureau of Dairy Industry.

The committee recognizes the fact that milk and cream sold for fluid consumption, with the exception of that in some of our smaller communities, is of good quality. We also recognize the fact that a large quantity of milk entering into the manufacture of dairy products is not of a satisfactory sanitary quality; and that improving the quality of such milk and cream is not only an extension problem, but one that is not receiving proper consideration.

Extension projects have been outlined for improving the quality of milk and cream. Many of these have been criticized on the basis that they were too complicated and involved too much time. It is the opinion of this committee, however, that in order for extension workers to accomplish worthwhile quality-improvement work they must first have a well-planned project and a definite goal in view.

B. F. Whitmore, dairy extension specialist of Illinois, has been operating an area plan of quality improvement for the past 18 months. The plan is similar to that advocated by your quality committee for the past 2 years. During this time a marked improvement in the quality of milk sold for all purposes has been accomplished. There has been a marked decrease in the quantity of Grade C milk and a marked increase in the quantity of Grade A milk produced, the grades being based on the methylene blue reduction test.

E19. Growth of Pennsylvania 4-H Club Calves. JOE NAGEOTTE, Pennsylvania State College.

Our dairy club members join a calf club to start a herd of purebred cattle and to learn how to care for a purebred herd. To reach this goal they need close supervision by the dairy extension specialist. The result of this supervision is a result demonstration on growing heifers. These demonstrations by club members have been one of our best methods to introduce improved practices in dairy herds. We dairymen have also learned from the club members what can be done and what cannot be done on farms. From this knowledge has evolved a method for growing out calves that may be of value.

Most members secure calves from 3 to 8 months old. They are fed hay, water, and a grain mixture containing 16 per cent protein and one teaspoon of cod liver oil a day. They are fed as much grain as they need to keep them in good growing condition until they are about 16 months old. Grain required varies from 2 to 8 pounds per day. The calves are not pastured their first summer and are not fed silage their first winter. At about 16 months of age the heifers are bred and then fed only roughage of hay and silage in winter or pasture in summer.

This method of feeding brings about a very rapid growth while the heifers are young. Where the schedule has been followed closely the heifers have shown the heart girth and height of withers of heifers several months older as shown by the standard rate of growth chart of Ragsdale, et al.

Figures have been collected from two clubs and will be presented by chart or slides.

E20. The Contribution of 4H Dairy Clubs to the General Dairy Extension Program. J. O. TRETSVEN, Montana State College.

One of the most important features of the Smith-Lever act provides for a system of itinerant teaching for farm boys and girls. It is a well recognized fact in teaching principles that a general educational program, as provided for by the Smith-Lever act is most effective by working with the farm youth. Every county agent who has had several years of experience in working with farm folks well knows the wisdom of this policy. The 4H Dairy clubs have contributed in many ways to the general dairy extension program.

1. The 4H Dairy clubs have in many cases been a means of getting adults interested in agricultural extension work. Parents who have been indifferent to or opposed to the work of county agents have become staunch supporters of the extension service through their son or daughter becoming a 4H Club member and in turn the parents have been greatly benefited by the service.

2. The instructions offered through the 4H dairy projects have not only resulted in developing the boys and girls to become more efficient in selecting, feeding and caring for their animals, but it has been a means of teaching better dairy practices in the community. The 4H dairy demonstration teams have taught, in a most effective manner, by actual demonstrations many valuable lessons on important phases of dairying.

3. The organization of dairy clubs have in many instances been instrumental in bringing into the community high class animals which in turn has been a big factor in the improvement of the cattle locally.

4. The training and experience offered the 4H Club members have created an interest in improving dairy cattle and a desire to go into the dairy business on a higher plane. In later years many of these 4H Club members

have developed into breeders of purebred cattle and in turn have been a source of good breeding stock in the community.

5. The 4H Dairy Club members learn the principle of cooperation in both work and play. A well organized dairy club may, through their own organization, be instrumental in uniting the dairy farmers of a community into essential cooperative organizations. Many former 4H Club members are, today the principal community leaders in their county.

No dairy extension program will be complete without a strong 4H Club program.

PAPER READ BY TITLE

———. **The Relation of the Posterior Hypophysis in the Maintenance of Lactation in Hypophysectomized Rats.** E. T. GOMEZ, U. S. Bureau of Dairy Industry.

Lactation which normally ceases immediately following complete hypophysectomy of lactating rats was maintained for periods ranging from 7 to 10 days by the simultaneous daily injections, beginning immediately after the operation, of adrenal cortical extract (eschatin), glucose solution and an aqueous suspension of anterior pituitary powder of proven lactogenic activity. The young of such animals, however, succumbed in 2 to 3 days, apparently of starvation, in spite of the fact that they suckled their mother's breast almost constantly and milk was present in quantities in the mammary glands. Successive litters introduced to hypophysectomized lactating rats immediately after the death of their own young likewise succumbed after a few days. Frequent inspections and autopsy of the young revealed complete absence of milk from the digestive tract.

Injections of posterior lobe extract (pituitrin) along with the daily "lactation maintaining" therapy of hypophysectomized rats permitted the young to get the milk from the mammary glands. By these treatments the period of the maintenance of lactation was extended for periods of over 28 days. Withdrawal of pituitrin from the daily therapy at any time during the course of the experiment was immediately followed by a rapid loss of body weight of the young terminating in death unless pituitrin injections were promptly resumed.

The average body weight of young reared by hypophysectomized rats was significantly less than that of young reared by control animals, including lactating rats subjected to sham operation, normals or those rats kept on limited daily food intake equivalent to that of hypophysectomized lactating rats. The average daily food intake of hypophysectomized rats was approximately 50 to 60 per cent below those of normals.

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A COMPARISON OF ALFALFA SILAGES PREPARED BY THE A.I.V. AND MOLASSES METHODS[1]

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INTRODUCTION

The large losses of nutrients which accompany ordinary haymaking, especially under unfavorable climatic conditions, have led to a search for a more efficient means of preserving forage. Of the various methods which have been tried, ensiling has received the most attention. At the present time several procedures appear to be practical for the ensiling of legumes and grasses. Two of these are the A.I.V. process, developed by A. I. Virtanen (1–2), which involves the addition of a mineral acid solution to the forage as it is ensiled; and the molasses method, apparently first used by Reed and Fitch in 1917 (3), and recently revived by numerous investigators (4–7).

Watson and associates (8–11) have made an extensive study of various methods for ensiling grassland products. Upon the basis of chemical analyses and feeding trials, they concluded that, while slightly lower losses of nitrogen are obtained with the A.I.V. method, the use of molasses is most applicable to English farm conditions. Davies (12) and other European workers (13) reached a similar conclusion from their experiments. Virtanen (14), however, reported the A.I.V. method as the more satisfactory. His conclusion was based upon the analyses of silages produced in small containers in the laboratory. Reports of success with both types of silage have also come from several investigators in this country (4–7, 15–17). The

Received for publication August 8, 1938. The delay in the publication of this paper was occasioned by an error in filing it in our office. We extend our apologies for this regrettable occurrence to the readers as well as the authors.—*Editor.*

[1] Cooperative experiment by the Departments of Biochemistry, Dairy Husbandry, and Agricultural Engineering. Various phases of the work have been done with the advice or supervision of Professors F. W. Duffee, C. A. Elvehjem, E. B. Hart, B. H. Roche and H. Steenbock. Credit is due Mr. G. M. Werner for direct supervision of the feeding of the cows and collection of many of the milk samples.

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earlier results obtained at this station with the A.I.V. method were reported in previous publications (16–17). Similar data comparing the A.I.V. and molasses methods are now available. The comparisons were made with respect to the preservation of forage nutrients, and to the quantity and quality of milk produced on rations containing the silages.

PREPARATIONS OF SILAGES

Each year the silages were prepared from first-cutting alfalfa in the early bloom stage. About 30 tons were ensiled by each method in 1935; 20 tons in 1936, and 23 tons in 1937. The method of preparation of the A.I.V. silage was the same as has been described previously (16) except that the alfalfa was chopped and the acid solution was pumped into the silo with a small centrifugal pump, and sprayed on the forage as it came from the blower pipe.

Commercial blackstrap molasses was used in the preparation of the molasses silage. In 1935 the molasses was diluted with an equal volume of water and applied in the same manner as the A.I.V. acid solution. In 1936 the lower half of the silo was filled in the same way, the upper half by applying the undiluted molasses to the alfalfa back of the feed roll of the ensilage cutter. The 1937 silage was also made with undiluted molasses. The application of undiluted molasses is simpler and requires considerably less labor.

The cuttings of alfalfa in 1935 and 1936 were at approximately the same stage of maturity although there was considerable difference in dry matter content. In 1937 the alfalfa was somewhat younger and low in dry matter. These differences provided a comparison of the two methods for ensiling forage of high and low moisture content; and also partially account for the large amount of A.I.V. acid solution required in 1936, 100 liters per ton, whereas 65 liters per ton were sufficient in 1935 and 80 liters in 1937. Although 100 pounds of molasses were used for each ton of alfalfa in 1935, barrel experiments which were run simultaneously (and also the results of other investigators) indicated that less was sufficient; accordingly, the rate was reduced to 65 pounds per ton in 1936 and 1937.

The large amount of drainage from both silos in 1935 and 1937 could not be measured accurately, since a considerable quantity of liquid seeped through the joints around the doors and was lost. Previous experiments had shown the losses of dry matter and nitrogen by drainage to be practically negligible (10). No drainage occurred from either silo in 1936.

ANALYSIS OF SILAGES

Five samples were taken from each silo during the feeding periods. The first was obtained after approximately three feet of silage had been removed, including about 6 to 12 inches of partially spoiled material on the surface. Subsequent samples were taken at about four foot intervals. Dry matter, pH, carotene, and ammonia nitrogen were determined immediately on all samples and two of the samples from each silo were analyzed for total nitrogen. In addition, fermentation products were determined upon the last samples obtained from the silos in 1935 and 1936. A summary of these results is presented in Tables 1 and 2.

TABLE 1

Chemical analysis of forage

Forage	Dry matter	pH	Total N (dry basis)	NH_3-N	Carotene (dry basis)
	per cent		*per cent*	*Total N per cent*	*Micrograms per gram*
1935					
Green Alfalfa	22.6		2.84	1.9	137
A.I.V. Silage	23.4	3.5	2.75	3.5	216
Molasses Silage	22.3	3.7	2.63	6.2	150
1936					
Green Alfalfa	30.9				195
A.I.V. Silage	25.4	3.8	2.35	5.4	164
Molasses Silage	28.2	4.3	2.13	9.5	91
1937					
Green Alfalfa	20.2				274
A.I.V. Silage	25.1	3.4	3.07	4.2	256
Molasses Silage	26.8	3.9	2.92	8.2	170

TABLE 2

Fermentation products of silage (per cent of dry matter)

Analysis	1935		1936	
	A.I.V. silage	Molasses silage	A.I.V. silage	Molasses silage
Volatile acids, as acetic	1.32	3.81	2.28	2.66
Acetic acid	1.32	3.81	1.89	2.44
Butyric acid	0.0	0.0	0.41	0.22
Non-volatile acids, as lactic	2.14	9.58	7.25	12.30
Lactic acid	0.71	8.15	3.98	8.12
Ethyl alcohol	0.21	0.77	0.32	0.69

Uniformly low values were found for pH and ammonia for all the A.I.V. silages and for the 1935 and 1937 molasses silages. In 1936, however, the upper half of the molasses silage was of only fair quality. The first sample had a pH of 4.6 and contained 21 per cent of the total nitrogen as ammonia. These values were lower in subsequent samples and the bottom half was of high quality with a pH of 4.1 and only 4.5 per cent of the nitrogen as ammonia. In spite of the variation in chemical composition, all of the silages seemed to be of good quality with respect to appearance and odor.

In 1935 and 1936, the carotene determinations were made according to a method similar to that of Peterson, Hughes and Freeman (17a). Both silages showed higher values in 1935 than the alfalfa which was used in their preparation, the values being 137 micrograms per gram for the green alfalfa, 216 for the A.I.V. silage and 150 for the molasses silage. In 1936, however, some carotene was lost from both silages, but especially from the molasses silage. Samples taken near the top of the molasses silo analyzed only 50 micrograms of carotene while those taken from the bottom half, where both pH and ammonia were lower, showed 150 micrograms per gram.

The observations of Quackenbush, Steenbock and Peterson (18) threw considerable doubt upon the accuracy of these figures. These investigators have shown that in carotene analysis the benzine or petroleum ether fractions of the pigments obtained from silages contain considerable amounts of carotenoids other than carotene; that this is especially true of silages prepared with mineral acids. Consequently in 1937, the carotene determinations were made by a modified procedure and carefully checked by chromatographic analysis. Again the A.I.V. silage showed consistently higher values than the corresponding molasses silage, although some carotene, approximately 13 per cent, had been lost in the A.I.V. silage. Forty-three per cent of the original carotene had been lost in the molasses silage.

Total nitrogen determinations showed slightly lower values for the molasses silages than for the corresponding A.I.V. silages. However, these figures must be considered as only approximations since there was considerable variation in individual samples and only two analyses were made upon each silage.

Fermentation products, especially non-volatile acids, were formed in larger amounts in the molasses silages than in the corresponding A.I.V. silages. However, the A.I.V. silage in 1936 contained larger amounts of total non-volatile acids and lactic acid than that prepared in 1935. Formation of butyric acid was completely suppressed in both silages in 1935. Although butyric acid was present in slight amounts in both silages in 1936, there was no objectionable odor.

In 1935 the temperatures were taken by means of thermocouples six feet below the surface and in 1936 and 1937 by thermometers suspended in pipes which were driven down 10 feet into the silage. The bulb of each thermometer was enclosed in a tube of water so that the reading did not change while the thermometer was being withdrawn and read. The curves obtained are shown in Figure 1. In all of the silages there was a rather abrupt rise during the first few days after ensiling although there was no excessive heating in any of the silages. The maximum temperature obtained is probably dependent to a large extent upon the dry matter content of the forage. In 1935 the silos were filled immediately after a heavy rain and very little heating occurred in either of the silages. The other two

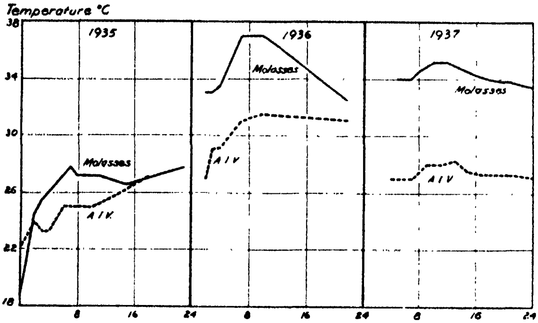

FIG. 1. Temperature changes in silage ten feet below surface.

years the temperatures of the A.I.V. silages were considerably below that of the molasses silages probably because respiration is stopped almost immediately after the acid is applied.

MILK PRODUCTION EXPERIMENTS

Cows from the University herd which had been receiving winter rations for about three months were divided into three lots approximately equal in

TABLE 3

Average daily feed consumption per cow (pounds)

Lot	Alfalfa hay	Corn silage	A.I.V. alfalfa silage	Molasses alfalfa silage	Grain mixture*
1935					
Control	10.7	32.0			10.0
A.I.V.	5.5	20.2	34.4		10.0
Molasses	5.4	18.9		36.4	9.9
1936					
Control	11.3	33.9			11.2
A.I.V.	11.3		30.9		11.0
Molasses	11.7			30.1	11.1
1937					
Control	7.6	45.7			10.0
A.I.V.	7.2		49.5		10.0
Molasses	7.2			49.9	10.0

*** Grain mixture:**

	Control lot	*Alfalfa silage lots*
	%	%
Ground corn	**61.9**	**69.6**
Ground oats	**24.7**	**27.5**
Linseed meal	**12.4**	
Bone meal		**1.9**
Iodized salt	**1.0**	**1.0**

In 1937, the grain mixture for the control lot contained 54.46 per cent ground corn, and 19.8 per cent linseed meal.

The cows on the A.I.V. ration received no salt but were fed 1 oz. of $CaCO_3$–Na_2CO_3 mixture (10: 3) per 15 pounds of silage.

weight, age, breed, stage of lactation, and milk and butterfat production. In 1935, each lot consisted of two Holsteins, two Guernseys, one Jersey and one Brown Swiss; in 1936, two Holsteins, two Guernseys and one Ayrshire, with the exception that a Brown Swiss replaced the Ayrshire in the molasses silage lot; and in 1937, two Holsteins, one Guernsey, one Brown Swiss and one Ayrshire. After the cows had been on experiment about six weeks in 1937 the Ayrshire in the A.I.V. lot had to be removed because of a badly infected udder. Since the production, weight, etc., of the Ayrshires in the three lots were similar they were removed from all three lots.

The composition of the rations is shown in Table 3. The hay used the first two years was western grown alfalfa of good quality and contained 27 and 50 micrograms of carotene per gram of dry matter in 1935 and 1936 respectively. In 1937, the alfalfa hay was quite leafy but rather discolored. It was probably more like that fed on the average farm and contained only 17 micrograms of carotene per gram of dry matter. The corn silages were those fed the University herd. In 1935 and 1936 they appeared to be of good quality, but there was considerable difference in their carotene content—37 micrograms per gram of dry matter in 1935 and 126 micrograms per gram in 1936. During the first two months of the feeding experiment in 1937 the corn silage was quite brown and contained only 22 micrograms of carotene. It was better in the lower part of the silo where the carotene ranged from 50 to 60 micrograms per gram of dry matter. The grain mixtures for the three lots were similar except that linseed meal was included in the mixture for the control lots to bring the protein content of the ration to the level of the legume silage rations. In 1937, when rather large amounts of corn silage and small amounts of alfalfa hay were fed to the control lot, it was necessary to include twenty per cent of linseed meal in the grain mixture.

In 1935, one-third of the corn silage and approximately half of the hay in the control rations were replaced by alfalfa silages in the A.I.V. and molasses lots. In this and the following years replacements were made on the basis of equivalent dry matter. In 1936, alfalfa silage replaced all of the corn silage in these lots. In 1937, the cows were encouraged to eat as much legume silage as possible by gradually decreasing the hay allowance. The average consumption was approximately 50 pounds per day although several animals ate 56 to 58 pounds. In the control ration the hay was also cut down and the corn silage increased so that all of the lots received approximately two-thirds the normal hay allowance.

There was no evident difference in the palatability of A.I.V. or molasses silages but they were not consumed as readily as corn silage. Some difficulty was encountered at first in getting the cows to eat large amounts of the legume silages but this was overcome by gradually substituting for the corn silage until the desired level was reached. As shown in Table 4, the cows gained in weight each year although no lot showed consistently greater gains than the others.

TABLE 4

Average milk production and changes in live weight (pounds per cow)

Lot	Daily milk production*	Decline in daily milk production*	Initial weight (lbs.)	Gain in weight
1935 (18 wks.)				
Control	27.8	6.2	1061.7	56.3
A.I.V.	29.6	5.0	1029.8	64.8
Molasses	29.2	3.5	1044.8	21.8
1936 (15 wks.)				
Control	33.4	7.9	1088.5	28.6
A.I.V.	29.3	5.3	1064.5	48.8
Molasses	31.2	6.1	1111.1	48.7
1937 (17 wks.)				
Control	28.3	2.1	1112.5	79.2
A.I.V.	28.0	4.0	1113.7	62.7
Molasses	28.2	1.4	1157.7	94.5

* 4 per cent fat corrected milk.

The milk production records are also presented in Table 4. During the three years, the milk and butterfat production was maintained almost equally well by all three lots although there is a slight advantage for the molasses silage lot over the three years.

CAROTENE AND VITAMIN A OF BUTTERFAT

Milk samples from each lot were obtained by pooling the milk from the morning and evening milkings. The carotene and vitamin A of the butterfat from these samples were determined spectroscopically by the method of Semb, Baumann and Steenbock (19). In 1935, only a single analysis at the end of the feeding period was made; in 1936 and 1937 several analyses were made at intervals throughout the period. The data are shown in Table 5. In 1935 and 1936 there was no marked difference in the carotene and vitamin A content of the butterfat from individual groups. The control lot was somewhat lower in both carotene and vitamin A in 1935 and slightly higher in both of these constituents in 1936. It was expected that the control lot in 1936 would produce butterfat of rather high vitamin A potency since the corn silage was of exceptionally good quality and high in carotene. However, at the end of the experiment the figures for the three lots were more nearly alike than the averages given in the table. During the last two months the carotene contents averaged 7.2, 7.5 and 6.1 and the vitamin A 8.5, 7.9 and 8.4 micrograms per gram for the control, A.I.V. and molasses lots respectively.

TABLE 5

*Carotene and vitamin A content of butterfat**

Lot	Number of determinations	Carotene	Vitamin A
		micrograms per gram	*micrograms per gram*
1935**			
Control	1	6.5	9.0
A.I.V.	1	7.6	11.0
Molasses	1	7.4	10.0
1936			
Control	6	7.2	8.7
A.I.V.	5	7.0	7.5
Molasses	5	5.6	7.4
1937			
Control	4	4.4	6.7
A.I.V.	4	7.7	11.1
Molasses	4	9.1	11.0

* Average of values obtained after cows had been on experiment three months.
** One determination made at the end of the feeding period.

In 1937 the differences between the control and the legume silage lots were considerable. The results are presented in detail in Figure 2. The vitamin A potency of the butterfat from the control lot fell rather rapidly until corn silage of better quality was used, about December 1, and then rose gradually until the end of the period. Both of the legume silage lots rose most rapidly during the first few weeks but continued to rise throughout the feeding period. The total vitamin A potency of the butterfat from the molasses lot reached a somewhat higher level than that of the A.I.V. lot although the cows in the A.I.V. lot were ingesting considerably more carotene. When the animals in the legume silage lots were placed upon the regular herd ration the carotene and vitamin A of the butterfat fell slowly. The 1937 results are indicative of what could be accomplished through the

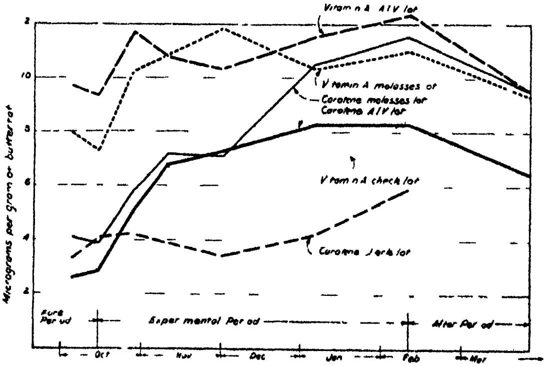

FIG. 2. Effect of ration on the carotene and vitamin A content of butterfat in 1937.

use of legume silages upon the average farm, since the hay and corn silage were quite similar to those ordinarily fed and the carotene and vitamin A contents of the butterfat from the control lot were similar to the average of winter milks which we have analyzed.

GROWTH PROMOTING PROPERTIES OF THE MILK

Composite milk samples were taken daily from each lot. These were mineralized (.5 mg. Fe, .05 mg. Cu, .05 mg. Mn per rat per day) and fed ad libitum to 30 gram male rats in growth experiments as described previously (16–17). The data are given in Table 6. In 1935, gains of 2.8 to 3.0 grams per day were made, which is normal for winter milks. In 1936, the growth was more rapid, approaching that obtained with summer milks. The excellent growth of the rats upon the control milk might be attributable to the high quality of corn silage which was fed. The rats receiving the A.I.V. milk showed somewhat greater gains in 1937. Unfortunately, too few rats were used in that year to warrant a definite conclusion.

TABLE 6

Growth of rats on mineralized milk (grams gain in 6 weeks)

Lot	1935	1936	1937
Control	128	162	106
A.I.V.	129	148	138
Molasses	120	148	119

Kohler *et al.* (20) have recently used the guinea pig as an experimental animal to determine the growth promoting properties of milks. None of the milks in 1937 were capable of supporting growth of guinea pigs. On all three milks, the animals lost weight rapidly and died in about three weeks.

DISCUSSION

Good preservation of alfalfa was accomplished by both the molasses and the A.I.V. methods of ensiling forage. As measured by the pH, ammonia and carotene data, the A.I.V. method showed some advantages over the molasses method. The carotene figures for the first two years are undoubtedly too high, especially for the A.I.V. silage, but in 1937 accurate determinations showed that the A.I.V. silage contained about 50 per cent more carotene than the molasses silage. The inferior quality of the top half of the molasses silage in 1936 may be attributed in part to the high dry matter content of the original forage. Satisfactory silage was obtained in the bottom of the silo where diluted molasses was used. Certain barrel experiments which we have run also indicate that for best results molasses should be diluted with material fairly high in dry matter.

Virtanen (22) has reported that a considerable portion of the added sugar in molasses silage disappears through alcoholic fermentation and is thus ineffective in preservation of the material. Our results do not show any more ethyl alcohol in molasses silage than is ordinarily encountered in corn silage (23).

Legume silage rations usually produced butterfat of higher carotene and vitamin A content than hay-corn silage rations. However, when the hay was of superior quality or the corn silage outstandingly high in carotene, the quality of the milk approached that obtained on alfalfa silage rations. Nevertheless, an increase in the carotene content of the ration was not followed by a parallel increase of vitamin A potency in the milk. This lack of response was especially evident in the A.I.V. groups. The A.I.V. silage contained appreciably more carotene than the molasses silage yet the milks produced therefrom were no more potent than those produced on the molasses silage. The data for 1937 show this most clearly, as in that year the carotene contents of the silages were determined by a more accurate method. The animals receiving the A.I.V. ration ingested approximately one-third more carotene than those in the molasses lot. Nevertheless, the total vitamin A potency of the butterfat from the A.I.V. lot was slightly lower than that from the molasses lot. Watson and Ferguson have also reported that A.I.V. silage was less efficient than either molasses silage or artificially dried grass in increasing the vitamin A potency of the butterfat.

The data on consumption, weight maintenance, and milk production are practically alike for the two alfalfa silages. From the point of view of initial cost of preservation there is probably little difference between the two methods. However, it should be pointed out that at least 75 per cent of the feeding value of the molasses is retained in the silage so that a large part of the initial cost is returnable. How much should thus be credited to the molasses method will depend of course on the feeding value assigned to molasses in comparison with equivalent feeds. Other items of cost in favor of the molasses method are the absence of license fees and cost of lime and soda. Taking various factors into consideration, especially the simplicity of operation, the molasses method appears to be preferable to the A.I.V. method. This conclusion is in agreement with that of Watson and associates who, after extensive experiments with the two methods, decided that for English conditions the molasses method is to be preferred.

Regardless of the method of ensiling, milk production should cost somewhat less with alfalfa silage than with corn silage because of the saving in high protein concentrates such as oil meal. This same advantage would of course be possessed also by alfalfa hay.

To the average farmer the greatest appeal of legume and grass silages lies undoubtedly in freedom from weather hazards during the harvesting of the crop. Absence of fire hazard is another advantage in making silage. On the debit side should be noted a somewhat greater labor cost as compared with hay-making and the necessity for more silos unless alfalfa replaces corn in the farm program.

SUMMARY

Alfalfa silages prepared by the A.I.V. and molasses methods were compared during three years. The comparison was based upon chemical analysis of the product and the quantity and quality of milk produced in feeding trials. Chemical studies showed no pronounced differences, although protein and carotene were better preserved by the A.I.V. method.

Good preservation of alfalfa containing 20 to 25 per cent of dry matter was obtained by both methods. Carotene and protein losses were somewhat larger when the forage contained 30 per cent dry matter.

Rations containing either A.I.V. or molasses alfalfa silage, or corn silage and linseed meal, were equally satisfactory for milk production.

The carotene and vitamin A contents of the butterfats produced on the legume silage rations were high but only in 1937 appreciably different from those produced upon the control ration. It is probable that under average farming conditions the vitamin A potency of the butterfat can be greatly increased by the use of legume silages.

The milks from the three lots had approximately the same growth promoting properties when tested on rats. All of the milks in 1936 were superior to most winter milks. None of the milks in 1937 would support growth of guinea pigs.

Molasses alfalfa silage appears to be equal to A.I.V. alfalfa silage, and conditions of farm practice probably make molasses silage more suitable for general use.

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THE DETERMINATION OF BLOOD PLASMA CAROTENE IN THE BOVINE USING A PHOTOELECTRIC COLORIMETER[1]

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The determination of the carotene content of blood plasma is of value in vitamin A studies especially when the entire food source of vitamin A is in the form of carotene as is usually the case with herbivorous animals.

Although there are many pigments in the blood stream the methods generally used for the determination of carotene exclude interference of the bile and respiratory pigments. The pigments extracted from blood serum or plasma by the methods which employ ethyl alcohol for the precipitation of the proteins and ethyl or petroleum ether for extraction, fall in the carotinoid class, the most important being the carotenes and xanthophylls.

Palmer (1) reported that in bovine serum, the carotinoids consist almost entirely of carotene with a very small fraction of xanthophyll. The carotinoids of horse serum also consist almost entirely of carotene whereas chicken serum contains mostly xanthophyll. In the case of human serum carotene accounts for most of the carotenoid fraction although some xanthophylls may be present. Gillam and Ridi (2) found only 5 per cent of the total carotinoids of bovine serum to be xanthophyll. By concentration of the carotene fractions from several liters of cow serum and separation by the adsorption method with alumina they found very small amounts of kryptoxanthin.

Since carotinoid pigments other than carotene are present in bovine and equine serum or plasma in only very small amounts, it would seem that the precipitation of the proteins by means of alcohol and the extraction of the carotene with petroleum ether offers a very simple method of analysis. In other types of blood in which significant amounts of other pigments might be present it would obviously be necessary to make further separations.

Various modifications of this procedure have been used by White and Gordon (3), Palmer and Eckles (4), Patchevsky (5), Daniel and Scheff (6), Gillam and Ridi (2), and Clausen and McCoord (7).

In general the methods used are more time consuming than necessary because they involve repeated extractions to remove all of the carotene. This is not necessary if application is made of the Nernst distribution law by the use of a phase separation. Moreover the availability of the photoelectric colorimeter has greatly improved the measurement of color. For

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most of the methods the various factors and limitations have not been studied so that this was one of the main objectives of this investigation.

EXPERIMENTAL METHODS

After trying several methods the following procedure was adopted. Twenty-five to 30 ml. of citrated blood was drawn from the jugular vein and centrifuged to obtain the plasma. Ten ml. of plasma was pipetted into 75 ml. test tubes and 15 ml. of 95 per cent alcohol added from a burette. The tubes were corked and shaken to precipitate the proteins. Five ml. of petroleum ether was then pipetted into the tubes and the tubes tightly corked and placed in cold running tap water having a temperature of about 10° to 12° C. After about five minutes the tubes were shaken vigorously for one minute and again placed in the cold water. This shaking procedure was repeated twice, the tubes being returned to the cold water each time to prevent the loss of petroleum ether. Since the tubes were at room temperature when they were corked a small vacuum was produced upon cooling which tended to tighten the cork in the tube so that there was practically no loss of the solvent.

After the third and last shaking each tube was filled with cold tap water to about 1½ inches from the top to facilitate the separation of the petroleum ether layer. The tubes were immediately recorked and placed in a refrigerator at about 10° below the temperature of the tap water. The tubes were left in the refrigerator for one to three hours to permit the petroleum ether layer to separate after which the carotene concentration was determined. If an emulsion was present in the petroleum ether layer, it could be eliminated in a few minutes by the addition of 0.5 ml. of 95 per cent alcohol. The alcohol was permitted to drop through the petroleum ether layer.

In order to establish the relationship between the amount of carotene extracted by the method just outlined and the total amount present in the sample a procedure was used which was identical with the previous one up to the point where water was added to the tubes. At this stage the tubes were centrifuged and the petroleum ether layer pipetted into a 25 ml. volumetric flask. An additional 5 ml. of petroleum ether was added to the tube and the tube was shaken for one minute. After cooling, the tubes were again centrifuged and the petroleum ether removed as before. This procedure was repeated five times and the extract made up to volume with petroleum ether. The fourth extract would usually be colorless so that it was reasonable to assume that five extractions achieved complete removal of the carotene.

THE PHOTOELECTRIC COLORIMETER

The recent development of the photoelectric colorimeter offers a physical means for measuring the density of color eliminating the shortcomings of the ordinary colorimeter which requires matching depth of color with the eye.

The general principles of the single cell photoelectric colorimeter used in this investigation are relatively simple. A beam of light from a small bulb is passed through a glass filter to limit the light to the desired range of the spectrum. The light then passes through an absorption cell containing the colored solution and falls on a photocell producing a deflection in a galvanometer. The amount of light transmitted through the colored solution is proportional to the ratio of the final to the initial galvanometer deflections. The concentration of the colored substance in the sample can then be read from a chart showing the variation of light transmission with concentration.

The instrument used was the Evelyn micro photoelectric colorimeter (8) in which small amounts of a solution may be used. In this particular instrument the light passes through an open type cell so that various depths of a solution may be used. The depths produced by 0.5 ml., 1 ml. and 2 ml. of solution were used in this study. The open type cell works satisfactorily with light passing up through it when liquids of a low surface tension are used. With liquids of high surface tension such as aqueous solutions, a glass plunger may be used to eliminate the surface tension effect.

Two filters were used in this investigation. The first one, 440, was quite dense so that it was necessary to burn the bulb at almost its maximum in order to get sufficient light through the cell. This made it necessary to replace the bulbs quite often. All the data reported in this paper except as indicated were obtained by the use of this filter. In order to overcome this difficulty a second filter, M 440, was obtained which was less dense and permitted burning the bulb at a much lower intensity. Both filters had maximum transmission of light at a wave length of 440 millimicrons.

The instrument was calibrated by preparing solutions of carotene* of varying concentrations in petroleum ether B. P. 40–60° C. and recording the galvanometer readings for the particular concentration and depth used. The carotene used was not a pure Beta carotene but previous experience had shown it to give curves almost identical with those of pure Beta carotene.

The calibration data may then be transferred to one cycle semi-logarithmic paper by plotting the concentration of carotene against the galvanometer readings. Tables may also be set up by first preparing the graph and interpolating for intermediate points. The latter method effects a large saving of time when considerable work is to be done. From the graphs or tables the concentration of carotene per ml. of blood plasma can be calculated.

EFFECT OF USING PETROLEUM ETHERS OF DIFFERENT BOILING POINTS ON THE AMOUNT OF CAROTENE EXTRACTED

One of the first points to establish was the relationship between the method in which some of the carotene was still present in the hypophase and the total amount present. In this connection it was expected that the boiling point of the solvent used would have some effect upon the concentration of the carotene because the greater solubility of the shorter chained hydrocarbons in alcohol-water solutions would reduce the volume of the epiphase. Consequently four petroleum ethers with boiling points of 20–40° C., 40–60° C., 68–70° C. and 73–76° C. were selected for the comparison.

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* Purchased from S. M. A. Corporation.

In the adopted method two ml. of the epiphase was pipetted into 10 ml. volumetric flasks and made up to volume with each solvent. In this way all dilutions were the same so that the galvanometer readings could be compared directly. Filter M 440 was used in collecting these data. The readings were taken with 1 ml. samples and the results are shown in Table 1.

It will be noted that the galvanometer readings for the two lower B. P. petroleum ethers are much lower than those obtained by the total extraction method indicating that per milliliter of extract the carotene content was higher. These readings were sufficiently different to give values 10 to 12 per cent too high. When the galvanometer readings were converted to carotene values the following factors were obtained: for 20–40° C., B. P. .894; 40–60° C., B. P. .913; 68–70° C., B. P. .988; 73–76° C., B. P. 1.001.

The differences of the means were tested for significance by the Fisher method (9). The *t* values for the differences between the means obtained by the total extraction method and the phase separation with petroleum ethers with boiling points of 20–40° C. and 40–60° C. were 9.8 and 8.0 respectively whereas the *t* value from Fisher's tables for 10 degrees of freedom is 3.169 at the one per cent point. These indicate highly significant differences. There was no significant difference when the higher B. P. petroleum ethers were used. The difference between the means of the two higher B. P. petroleum ethers was significant. The *t* value was 2.91 as compared with a value of 2.23 at the 5 per cent point for 10 degrees of freedom.

It was observed that solvents of differing boiling points gave different center settings. For instance, a petroleum ether with a boiling point of 20–30° C. gave a center setting of 48 whereas one with a boiling point of 73–76° C. gave a center setting of 52.4. However, when solutions of carotene of the same concentration were made with the different solvents the same galvanometer readings were obtained. Consequently the calibration data obtained with a solvent of one boiling point may be used with a solvent of a different boiling point.

EFFECT OF TEMPERATURE DURING EXTRACTION

In order to study the effect of temperature on the amount of carotene extracted by the solvent, determinations were made at 3 different temperatures using petroleum ether with boiling point of 40–60° C. One sample was extracted at room temperature, 25° C., one at that of tap water (10° C.) and one at − 24° C. In the latter case the proteins were precipitated

TABLE 1

Effect of boiling point of solvent upon carotene extraction

Sample No.	Total extraction 20–40° C.	Adopted method			
		20–40° C.	40–60° C.	68–70° C.	73–76° C.
	Galvanometer readings				
1	67.8	65.5	64.5	68.7	68.5
2	28.5	25.8	26.0	28.9	29.4
3	79.5	77.8	77.8	79.8	80.0
4	71.5	69.0	70.5	71.0	72.1
5	60.0	58.0	59.0	60.5	61.7
6	70.5	68.0	69.1	70.7	71.2
7	63.7	59.0	61.0	63.0	62.8
8	19.5	17.0	17.5	19.1	19.5
9	27.5	24.5	24.8	26.8	27.0
10	63.0	61.2	61.5	63.4	63.8
11	32.5	28.5	29.0	31.2	31.4

at room temperature, petroleum ether added and the tubes kept at −24° C. for about one-half hour. They were then shaken for three one minute periods, after which water was added with a temperature of about 0° to 1° C. The tubes were left in the cold until the petroleum ether layer had separated. The determination of the carotene was made at room temperature.

The results shown in Table 2 indicate a correlated agreement at the various temperatures. Using the statistical treatment previously mentioned the *t* value for the difference between the means at 25° C. and 10° C. was 4.66, for 10° C. and −24° C., 5.36 and for 25° C. and −24° C., 7.04. According to Fisher's tables the *t* value for 12 degrees of freedom at the one per cent point should be greater than 3.055 so that the means at the various temperatures are significantly different from each other.

TABLE 2

Effect of temperature of extraction upon galvanometer readings

Sample No.	+25° C.	+10° C.	−24° C.
	Galvanometer readings		
1	30.0	31.2	35.0
2	24.0	23.8	28.0
3	82.5	83.3	85.0
4	18.3	19.0	20.5
5	23.5	24.1	25.5
6	19.5	20.9	22.8
7	75.8	75.8	78.0
8	75.0	75.4	77.0
9	29.0	29.5	30.4
10	34.0	34.8	35.0
11	35.0	36.1	37.3
12	84.0	84.5	85.3
13	77.5	77.6	78.4

THE EFFECT OF THE TEMPERATURE OF THE PETROLEUM ETHER EXTRACT AT THE TIME OF PIPETTING INTO THE ABSORPTION CELL

Some of the solutions used to standardize the instrument (crystalline carotene dissolved in petroleum ether B. P. 40–75° C.) were cooled to below − 10° C. The temperature of the solution was allowed to increase and 1 ml. portions pipetted into the absorption cells at − 10, 0, + 10 and + 20° C. respectively. Measurement of the carotene was made immediately after each transfer. The results showed that the readings at the various temperatures checked quite closely.

EFFECT OF USING VARIOUS AMOUNTS OF ALCOHOL FOR PRECIPITATION OF PROTEINS

In order to study the effect of adding various amounts of alcohol, 5, 10, and 15 ml. of alcohol were added to triplicate samples of plasma. The results showed that for the 10 and 15 ml. amounts of alcohol the galvanometer readings checked fairly well whereas in the case of the addition of 5 ml. the results were less consistent. It was also noted that in the 5 ml. tubes an emulsion was often present in the petroleum ether layer.

EFFECT OF TIME OF SHAKING

Triplicate samples were prepared and the length of time of shaking the tubes was varied using 3, 2 and 1 minute periods. The results indicated fairly good agreement between the different times used although the 2 and 3 minute periods gave the most consistent values.

EFFECT OF ADDING ALCOHOL TO PETROLEUM ETHER LAYERS

Since it was necessary in some of the samples to add 0.5 ml. alcohol in order to clear up an emulsion in the petroleum ether layer it was decided to determine whether the use of larger amounts of alcohol would have any effect on the galvanometer reading.

Triplicate samples were prepared and previous to the transfer of the petroleum ether extract to the absorption cell 0.5 ml. and 3 ml. portions of alcohol were added to each set whether an emulsion was present or not. The addition of alcohol had no measurable effect on the galvanometer readings. It was noted, however, that alcohol added when the higher boiling petroleum ethers were used, caused a cloudiness to develop in the epiphase which cleared up only after standing for several hours.

RELIABILITY OF THE METHOD

In order to determine the dependability of the method frequent analyses were made of the samples over a considerable period. The blood was centrifuged immediately after collection and plasma stored in the refrigerator at a temperature of about 2.0° C. Ten ml. of plasma was removed at intervals and the carotene content determined. This was done 11 to 12 times on each sample over a period of 23 days with filter 440 and over a period of 15 days with filter M 440. Petroleum ether with a boiling point of 40–75° C. was used. The results are shown in Table 3 in which the means, extremes, standard deviations and coefficients of variation are given for each sample.

With filter M 440 the greatest deviation from the mean in 107 determinations exclusive of those obtained with sample C 118 amounted to 7.7 per cent of the mean. With filter 440 of 83 determinations excluding those of A 1, C 118 and C 245, the greatest deviation amounted to 13 per cent of the mean. With these exceptions the results were subject to little variation from day to day.

TABLE 3

Showing variations with statistical constants relative to the determination of carotene in blood plasma

Animal No.	Det. made	Micrograms carotene per ml.				Coef. of Var.	Mean Gal. reading
		Mean	Extremes		Stand. Dev.		
			Upper	Lower			
			440 Filter				
			Extract read directly				
A 1	12	0.052	0.092	0.025	±.027	51.9	96.8
G 33	12	0.57	0.65	0.53	±.033	5.8	64.4
C 118	12	0.09	0.12	0.06	±.016	17.7	93.6
A 22	12	1.84	1.92	1.70	±.069	3.8	32.5
A 14	12	2.07	2.21	1.94	±.083	4.0	23.4
C 245	12	2.78	2.90	2.44	±.192	6.9	16.4
			Extract diluted 1–10				
A 15	12	4.56	4.86	3.97	±.218	4.8	70.1
A 7	12	6.94	7.10	6.45	±.187	2.7	59.1
A 18	12	7.09	7.60	6.25	±.380	5.4	58.4
D 11	11	9.17	9.55	8.75	±.310	3.4	50.2
			M 440 Filter				
			Extract read directly				
C 118	12	0.15	0.17	0.14	±.009	5.7	90.0
G 33	12	0.52	0.56	0.49	±.021	4.0	69.6
A 22	12	2.21	2.31	2.12	±.064	2.9	27.3
A 14	12	2.29	2.39	2.17	±.062	2.7	26.4
A 26	12	2.68	2.76	2.62	±.046	1.7	22.1
			Extract diluted 1–10				
C 245	12	2.61	2.69	2.49	±.059	2.3	83.3
D 5	12	4.67	4.87	4.46	±.108	2.3	72.0
A 15	12	4.84	4.91	4.70	±.066	1.4	71.2
A 18	12	4.85	4.93	4.70	±.075	1.6	71.2
D 11	10	6.55	6.97	6.09	±.295	4.5	64.1

DISCUSSION OF RESULTS

The results obtained by using solvents of differing boiling points in relation to the total amount of carotene present are very interesting. The data shown in Table 1 indicate quite conclusively that different results may be obtained with solvents of different boiling points. With the lower boiling point petroleum ethers, lower galvanometer readings or higher carotene values were obtained. The differences between the means were statistically significant. With the higher boiling point petroleum ethers the galvanometer readings were very similar.

It must be recognized at the outset that several factors may affect the distribution and concentration of the carotene between the two phases, the alcohol-plasma-water on the one hand and the solvent on the other. One important factor is the miscibility of the two phases and another the distribution ratio of carotene between the two phases. A third is the effect of temperature on each of the others.

The results may be explained by the operation of these factors. In the case of a petroleum ether having a relatively high solubility in the hypophase the volume of the epiphase would be correspondingly low and the concentration of carotene per unit volume relatively higher. The greater solubility of the low boiling point ethers in the hypophase is due to the shorter chains of smaller hydrocarbon molecules. Seidell (10) gives data showing that pentane is considerably more soluble in an alcohol water solution than is hexane, heptane, or gasoline. The petroleum ether with a boiling point of 20–40° C. is probably mostly pentane. Data were not found showing the solubility of these various solvents in a 20–30 per cent alcohol solution which is approximately the concentration after the addition of water in the method used. However, such data would probably be of little value in a system where blood plasma was introduced since the fatty constituents of the plasma would undoubtedly change the solubilities from that of a pure system.

That the amount of carotene extracted with petroleum ether was less than the total amount was shown by the fact that if the hypophase was separated from the epiphase and the epiphase made up to the same volume as in the total extraction method, the galvanometer reading would be larger indicating a lower concentration of carotene. One factor which probably caused the retention of some of the carotene in the hypophase was the presence of some petroleum ether in this phase.

In the case of the petroleum ether with a boiling point of 73–76° C. the galvanometer readings were about the same as for the total extraction.

The results from Table 1 indicate that if petroleum ether is used in a phase separation of the carotene from an alcohol-plasma-water system a factor must be obtained for each petroleum ether with a different boiling point. The results further indicate that if petroleum ether B. P. 73–76° C. is used no factor is necessary since the results are essentially the same as those obtained by the complete extraction method. However, when emulsions develop with the higher boiling petroleum ethers so that it is necessary to add alcohol, the added alcohol causes a clouding of the epiphase making it necessary to let the sample stand for sometime before reading. When the readings are desired immediately, it would be advantageous to use a lower boiling petroleum ether and employ a factor.

When the effect of the temperature of extraction was studied, the results in Table 2 show that the concentration of carotene in the epiphase was greater at + 25° C. than at + 10° C. or − 24° C. Their means were shown to be statistically different from each other. These results are probably explainable on the basis of the solubility of the petroleum ether in the hypophase at the different temperatures used. According to Seidell (10) 80 per cent alcohol will take up about 10 per cent by weight of pentane at − 30° C. and 21 per cent at + 15° C. No data were given by Seidel at the alcohol concentration used in these studies but as stated before the blood plasma constituents would probably alter the miscibilities from that of a pure system.

The temperature of the extract at the time it was pipetted into the absorption cell had no measurable effect on the galvanometer readings. It is more than likely that due to the small amount of the extract used for the determination and the considerable contact with the pipette and absorption cell fairly uniform temperatures are attained at the time the readings are made regardless of the initial temperature of the extract.

The use of 10 cc. of alcohol for precipitation of the proteins gave as good results as 15 cc. When 5 cc. was used the results were not so consistent. It was found, also, that when 15 cc. of alcohol was used there was less tendency for emulsions to form.

The study of the influence of the amount of shaking indicated that greater consistency in results were obtained with two to three minutes. Although the time of shaking is a relative matter between different individuals proper mixing of the solvent and alcohol-plasma-phase is absolutely necessary in order to get consistent results. Shaking should not be so violent as to cause the formation of an emulsion. The presence of an emulsion in the lower portion of the petroleum ether phase had no effect on the readings. The use of alcohol to clear up an emulsion which might be present also had no effect on the results.

The results in Table 3 show that the method used is consistent from day to day when proper precautions are taken. The data show that there are but small variations from the mean and indicate that the method is quite reliable. The greatest accuracy was apparently obtained with filter M 440 with which the greatest coefficient of variation was 5.7. In this case, however, the mean galvanometer reading was 90.0. Although it is usually desirable to keep the galvanometer readings between 20 and 90, greater accuracy is probably attainable within a still smaller range such as 30 to 80.

In the case of filter 440 the two greatest coefficients of variation were 51.9 and 17.7. In both instances the galvanometer readings were above 90 which probably accounts for the large variations. The next largest coefficient of variation of 6.9 was obtained when the galvanometer readings were below 20.

An examination of the daily readings from the detailed data from which Table 3 was made further shows that plasma stored at 2° C. did not decrease in its carotene content. As a matter of fact, plasma samples stored as long as six weeks have maintained their carotene content. Studies on whole blood indicated that as long as hemolysis did not occur there was no decrease in carotene.

These studies show that carotene can easily be determined by the phase separation from an alcohol-plasma-water system by means of petroleum ether. The method is simple and many determinations can be made in one day. The results further show that two factors must be taken into consideration, one the boiling point of the solvent and the other the temperature of extraction. These two factors should be standardized. After standardization a comparison should be made with results obtained by a complete extraction of carotene and if necessary a factor calculated. The use of petroleum ether with a boiling point of 73–76° C. and extraction at a temperature of 10–12° C. obviates the use of a factor.

With some determinations a K value is obtained which may be used for calculation purposes when the calibration data forms a straight line plotted on semi-logarithmic paper. However, since the data for carotene do not quite give a straight line it was felt that the use of the plotted curve directly was more accurate, and more rapid.

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SUMMARY AND CONCLUSIONS

1. A simple phase separation of carotene from bovine plasma has been outlined.

2. When petroleum ether is used as the solvent the boiling point and temperature of extraction are important factors in the amount of carotene extracted.

3. When petroleum ether with a boiling point of 73–76° C. was used and the extraction carried out at 10–12° C. the epiphase gave true carotene values.

4. When the phase separation method is used, the relationship of the

amount of carotene in the epiphase to the total amount present in the sample should be established.

5. The temperature at time of pipetting into the absorption cell under ordinary laboratory conditions had no measurable effect on the galvanometer readings.

6. The use of 15 ml. of a 95 per cent ethyl alcohol for precipitation of the proteins from 10 ml. of plasma was found to be advantageous.

7. Shaking the tubes for two to three minute periods gave more consistent results than shaking for one minute.

8. The addition of 0.5 ml. of alcohol to destroy emulsions had no effect on the determination.

9. The method was shown to give consistent results from day to day.

10. Plasma stored at 2.0° C. showed no loss of carotene over a period of three weeks.

11. The use of the phase separation method in conjunction with the photoelectric colorimeter offers a simple and rapid procedure for the determination of carotene in bovine blood plasma.

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EFFECT OF PASTURE UPON THE CAROTENE CONTENT OF BLOOD PLASMA OF THE BOVINE

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Palmer and Eckles (1) demonstrated in 1914 that there was a relationship between the carotene content of the feed consumed and the carotene content of the blood serum. Gillam and Ridi (2) reported the average carotene content of blood sera of cows on winter feed to be 0.4 mg. per 100 ml. as compared to a value of 1.11 on summer feed. Semb, Baumann and Steenbock (3) found that the feeding of A.I.V. silage to 12 cows on winter feed produced an increase from an average value of 5.2 micrograms of carotene per ml. of plasma to 8.8 micrograms in 6 weeks. Since the data in the literature are rather limited as to the effect of pasture on the carotene content of the blood plasma of the bovine, it was decided to study this effect.

EXPERIMENTAL

In the spring of 1937 and 1938, just previous to pasture season, cows producing varying amounts of milk, and heifers were chosen from the Brown Swiss, Holstein, Ayrshire, Jersey and Guernsey breeds of the college dairy herd. In 1938 a few dry cows were included in the breed groups. Blood samples were drawn from the jugular vein at various periods previous to and after turning to pasture. Plasma carotene determinations were made according to a method (4) previously outlined. Extraction was made with petroleum ether B. P. 40–60° C. For this solvent the factor .913 was used as explained in the previous paper. The results in 1937 were obtained by the use of the Lovibond Tintometer whereas those in 1938 were obtained by the use of a photoelectric colorimeter.

In 1937 the cows were turned to pasture on May 10th and the heifers on May 17th. In 1938 the cows and heifers were turned out on various dates from April 19th to May 14th, and the nature of the pasture varied to some extent. However, at this season of the year all plants contain large amounts of carotene so that the types of the pastures themselves should have played little part in affecting the level of blood carotene. In 1938 the cows were fed some silage after turning to pasture because of the necessity of emptying a silo.

Blood samples were also drawn from a Holstein cow which had been on a low carotene ration but had received vitamin A by the daily administration

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TABLE 1

Carotene content of blood plasma of cows before and after turning to pasture in 1937

Animal No.	Age yrs.	Daily milk lbs.	Micrograms per ml.				
			Before pasture		After pasture		
			10 days	5 days	2 days	9 days	16 days
			Brown Swiss				
239	5	59	2.6	2.6	4.6	9.1	17.3
238	5	43	3.2	3.2	4.1	8.2	15.5
230	3	32	3.4	4.6	4.3	10.0	16.4
240	5	27	2.6	2.7	3.4	10.0	13.2
			Holstein				
197	5	72	3.2	3.7	4.6	10.0	17.1
253	3½	54	3.0	4.1	6.4	8.2	19.6
254	3	38	3.2	2.8	4.6	11.0	17.0
186	7	36	3.8	3.0	3.7	8.2	11.9
207	5½	8	2.1	2.5		2.5	6.7
			Ayrshire				
134	6	67	2.8	2.3	6.4	10.0	22.8
142	3½	46	5.4	4.1	8.2	11.0	21.0
150	2½	43	2.1	2.2	4.3	10.0	20.1
			Jersey				
116	3	29	4.8	4.1	7.3	19.2	23.7
113	3	26	5.1	5.8	8.2	20.1	20.1
115	3	23	4.8	3.7	7.3	15.5	21.0
107	4	22	4.1	3.2	7.3	12.8	22.8
95	5½	17	3.3	3.7	7.3	13.7	15.5
			Guernsey				
4	11½	42	6.8	5.0	10.0	19.2	28.8
42	3¼	38	8.7	7.5	11.0	24.6	28.8
36	3	27	6.4	6.4	10.0	21.9	25.6
48	2½	27	6.8	4.3	6.4	14.6	27.4

of 40 ml. of cod liver oil. This cow was three years old and had been raised on skim milk, corn starch, bran and cod liver oil with wood shavings as a roughage. She was given 40 pounds of fresh cut alfalfa in the morning at the time this experiment was carried out and had consumed about one-half of it during the first half hour. Each day thereafter she consumed about 80 pounds per day. This freshly cut alfalfa contained about 190 micrograms of carotene per gram on the dry basis so that the intake of carotene was about 2 grams per day. Blood samples were drawn every hour for 19 hours and then intermittently up to 168 hours after the feeding of freshly cut alfalfa hay. In this way the first effect of the consumed carotene on the level of plasma carotene was obtained.

RESULTS

The results obtained are shown in Tables 1 to 4. In Table 3, which gives the 1938 results, the last sample was drawn at periods varying from one day to a week before the animals were placed on pasture and those taken previously were drawn at weekly intervals.

The animals of each breed have been arranged according to decreasing milk production in Tables 1 and 3. The figure for milk production was the approximate amount after one week on pasture. The breeds have also been arranged approximately according to the increasing carotene content of the plasma.

An examination of the results in Tables 1, 2 and 3 shows that there are two factors which control directly or indirectly the level of plasma carotene, one, the breed, and the other, the amount of milk being produced or the stage of lactation.

The results show that the breeds rank in total amount of plasma carotene approximately in the order in which they are listed in the tables, that is, the Brown Swiss plasma contained less carotene under the same conditions than

TABLE 2

Carotene content of blood plasma of heifers before and after turning to pasture in 1937

Animal No.	Age yrs.	Before pasture		After pasture	
		10 days	3 days	4 days	10 days
		Brown Swiss			
233	2	1.3	1.6	4.5	3.2
234	1¾	1.6	0.8	6.4	4.3
		Holstein			
265	2	1.1	1.6	4.1	3.7
262	2¼	1.1	2.5	2.7	2.8
		Ayrshire			
156	1½	1.0	0.6	6.7	6.7
154	1¾	2.5	1.4	6.0	6.4
		Jersey			
80	1½	2.5	1.3	8.2	11.0
84	1¼	3.2	1.6	11.9	11.0
		Guernsey			
55	1½	3.3	3.2	8.7	16.4
54	1¾	4.1	2.8	14.6	21.9

TABLE 3

Carotene content of blood plasma of cows and heifers before and after turning to pasture in 1938

Animal No.	Age yrs.	Daily milk lbs.	Micrograms per ml.					
			Before pasture		After pasture			
			7–14	1–7	7 days	14 days	28 days	56 days
			Brown Swiss					
239	6	55	2.9	2.7	9.1	8.7	11.8	14.3
238	6	43	3.3	3.3	9.9	11.5	13.8	13.9
240	6	21	3.7	3.7	8.7	10.0	12.8	12.7
230	4	13	3.0	3.0	6.0	6.8	9.6	......
303	1½	....	2.1	2.0	3.3	3.9	4.1	....
305	1½	.....	1.4	1.7	1.7	2.6	2.6	. ..
			Holstein					
258	3½	60	2.9	2.8	.....	11.1	14.4	20.5
186	8	41	4.2	3.8	..	12.3	15.5	15.8
256	4	22	2.3	2.4	5.2	6.4	7.9	.
220	6	12	2.7	2.9	5.7	6.4	8.8	6.6
278	1½	.....	2.3	2.1	5.0	7.1	7.0	.....
279	1½	....	2.5	2.6	6.1	7.1	7.4	....
			Ayrshire					
156	2	37	4.4	5.0	10.9	12.1	14.4	16.5
157	2	36	3.0	3.1	10.0	12.7	13.7	19.1
141	5	10	.....	3.2	7.1	9.6	10.0	.....
134	7	.....	..	2.3	6.8	8.1	10.2	5.0
161	1¾	.....	2.5	3.6	5.8	6.0	6.3	.....
160	2	....	1.9	1.9	3.5	4.8	5.8	....
			Jersey					
117	4	36	4.0	4.8	15.7	16.7	19.7	23.3
111	5	31	3.1	4.4	10.3	10.4	14.2	13.7
116	4	14	.....	3.7	11.6	15.1	17.7	13.2
114	4	......	2.5	3.0	8.7	8.7	12.8	4.9
90	1½	.....	1.6	3.2	5.2	7.6	13.1	....
84	1½	......	2.5	1.8	5.3	6.0	8.9	......
			Guernsey					
41	4½	36	6.6	6.8	....	20.8	25.6	23.6
47	3½	32	7.2	7.5	....	28.6	32.0	21.9
44	4½	17	......	5.8	16.6	19.1	21.1	18.3
25	6	......	......	5.8	9.1	14.7	19.0	24.3
3	1½	......	3.7	4.0	9.5	12.1	17.3	....
6	1¼	......	5.5	5.5	12.2	16.1	16.1	....

the Jersey and Guernsey breeds. There appeared to be little difference between the Brown Swiss, Holstein and Ayrshire breeds, although the Ayrshires in a few instances gave higher values.

With respect to milk production within the breed the higher the production the higher the carotene content of the plasma after turning to pasture. The heifers were still somewhat lower and in Table 3 are shown to be lower than the dry cow included in three of the breed groups. However, while the animals were on winter feed this relationship did not hold.

TABLE 4

Carotene content of blood plasma of a cow fed freshly cut alfalfa

Hours after feeding	Micrograms/ml.	Hours after feeding	Micrograms/ml.
0	0.19	14	0.26
½	0.21	15	0.33
2	0.21	16	0.30
3	0.20	17	0.31
4	0.20	18	0.33
5	0.25	19	0.33
6	0.18	22	0.49
7	0.22	28	0.64
9	0.18	36	1.21
10	0.22	48	2.74
11	0.22	120	6.85
12	0.25	168	8.49
13	0.24		

The results in Table 4 show that the first rise in plasma carotene which might be attributed to the high intake of carotene was at 15 hours. This was followed by a very definite rise at 22 hours. The feces at the end of 18 hours were definitely green so that the rise at 15 hours was probably significant. The rise in plasma carotene continued until at the end of the first week (168 hours) the plasma carotene level was about the same as that of Holstein cows after one week on pasture.

DISCUSSION

The results clearly indicate that there are distinct breed differences in the level of plasma carotene under both winter and summer feeding conditions. The breeds ranked according to increasing plasma carotene content in the following order: Brown Swiss, Holstein, Ayrshire, Jersey and Guernsey. However, the differences between the Brown Swiss, Holstein and Ayrshire breeds were small. These results show a relationship with those obtained by other investigators in which the butterfat of the Jersey and Guernsey breeds was found to contain more carotene than that of the Holstein and Ayrshire breeds under the same conditions. In other words, the higher the carotene content of the plasma the higher is the carotene content of the butterfat produced, although the total vitamin A activity of the butterfat of the different breeds remains essentially the same under similar feeding conditions. One might reason, therefore, that the true vitamin A content of blood plasma varies inversely with the carotene content under the same feeding conditions.

Accordingly the true vitamin A content of Holstein plasma would be higher than that of the Guernsey whereas the carotene content would be correspondingly lower.

The reasons for breed differences in these respects are not clear. It may be that the carotene concentration of the blood plasma is governed by differences in absorption or by variations in ability of the liver to convert the carotene to vitamin A. Fasold and Heidemann (5) reported that in goats 8 days after thyroidectomy, the milk fat which is normally white, had a yellow color. The yellow color was shown to be produced by carotene. Murlin (6) has recently reviewed the relationship between vitamin A and thyroxin. Many reports are also present in the literature that diabetics show an increased carotene content of the blood serum (7).

There also appears to be a definite relationship within the breed between milk production and the total increase in plasma carotene after turning to pasture. In other words, the greater the milk production the greater the increase in plasma carotene. The reason for such an increase is not apparent. One might suggest that it was due to greater total intake of carotene associated with the consumption of larger amounts of pasture for the heavier producing cows. This reasoning has some support in the fact that on winter feed the plasma carotene values for the milking cows do not vary with milk production since it is the usual herd practice to feed about the same amount of roughage to all cows.

The plasma carotene values for the heifers of each breed under similar conditions were definitely lower than the values for the milking cows of the same breed.

The results for the two years are on the whole in agreement although those of 1937 were obtained by the use of the Lovibond tintometer and are therefore much less accurate. Higher plasma values for both Ayrshire cows and heifers were secured in 1937 than in 1938. The rise of plasma carotene in 1938 appeared to be a little slower than in 1937. This was probably due to the fact that the cows received some silage in the barn and did not consume as much pasture as in 1937.

It is not certain whether maximum values were obtained in these results although in some instances there was some decrease after 56 days. However, it would be reasonable to assume that maximum values had been reached after this period.

The results obtained with the cow fed the freshly cut alfalfa showed that the first rise occurred approximately 15 hours after the initial consumption of the alfalfa. The rate at which the carotene rises in the blood plasma probably fluctuates with the rate of food passage which varies considerably (8). Using these data in conjunction with those in Tables 1, 2, and 3 one would be led to the conclusion that the greatest rate of rise in carotene concentration of the blood plasma occurred during the first week, after which further increases were at a slower rate.

SUMMARY AND CONCLUSIONS

1. Different breeds kept under similar conditions of feeding ranked in increasing order of plasma carotene values as follows: Brown Swiss, Holstein, Ayrshire, Jersey and Guernsey. There was little difference between the first three.

2. Within the breed there was little difference in the level of plasma carotene values among cows producing milk under winter conditions.

3. Heifers within the breed had definitely lower plasma carotene values than producing cows under both winter and pasture conditions.

4. Cows producing the larger amount of milk within the breed in general showed a greater increase in the level of plasma carotene when they were turned to pasture.

5. One cow with a very low initial plasma carotene value showed an increase within 15 hours after being fed freshly cut alfalfa.

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THE USE OF STEAPSIN IN THE MANUFACTURE OF BLUE CHEESE

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Blue cheese made from normal cows' milk, even under the most favorable conditions, must be held approximately six months before enough of the characteristic flavor has developed for the cheese to be marketable. The length of the ripening period is a serious deterrent to extensive commercial manufacture of the cheese in this country.

Since the work of Currie (1) fat hydrolysis by a water-soluble lipase produced by the mold, *Penicillium roqueforti*, has been considered the chief factor in the development of the typical flavor of Roquefort-type cheeses. Lane and Hammer (2) were able to shorten the ripening period by homogenization of the milk from which the cheese was manufactured. They attributed the quicker ripening of homogenized milk cheese to the natural milk lipase, the action of which is accelerated by homogenization. They found it necessary to homogenize the milk at temperatures below 115° F. if the benefits of homogenization in shortening the ripening period were to be realized to the fullest extent. This is compatible in some respects with the work of Sharp and de Tomasi (3) showing that non-lactic acidity and bitterness did not develop in cream if the milk before separation was flash heated to 110° F. (43.3° C.). It was found, however, that if the cream was homogenized the milk had to be heated to 140° F. (60° C.) in order to prevent the lipase action in the cold cream.

Various investigators have shown that the ripening of cheese of the Cheddar type could be hastened by increasing the amount of rennet used. Orla-Jensen (4) found that in Emmental cheese, trypsin caused much more rapid proteolysis than either pepsin or galactase. Wojtkiewicz and Inikkoff (5) attempted to hasten the ripening of cheese by adding pepsin, trypsin, erepsin and rennet separately and in various combinations. Rennet and trypsin were found to hasten the ripening of the cheese. The cheeses to which trypsin was added had a bitter flavor. Better results, from a flavor standpoint, were secured when pepsin was added in combination with trypsin, although some bitterness was evident. Freeman and Dahle (6) experimenting with the ripening of Cheddar cheese found that additional amounts of pure rennin increased the rate of proteolysis. This was also true for pepsin, particularly at the beginning of the ripening period, and an aged product with an appreciably higher flavor score was obtained. Trypsin

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markedly increased the rate of proteolysis during the early stages of the ripening period, but cheese with a lower flavor score was obtained.

EXPERIMENTAL

Since fat hydrolysis appears to be essential for the development of the characteristic flavor in the Roquefort-type cheese, the addition of enzyme preparations containing lipase appeared to offer some promise of shortening the ripening period.

In March, 1935, a small quantity of milk was manufactured into Blue cheese with commercial steapsin (Pfanstiehl) added to the curd at the rate of two grams per 100 pounds of milk. The resulting cheese developed the characteristic flavor of a Roquefort-type cheese within three months. The control cheese did not have the same intensity of flavor even after six months. Subsequently, more than twenty-five batches of cheese have been made with steapsin added in various amounts, either to the milk before renneting or to the curd before hooping, for all or part of the cheese in each batch. The quantity of the enzyme preparation varied from 0.1 gram to 2 grams per 100 pounds of milk.

The addition of steapsin to the milk or to the cheese curd before hooping at the rate of 1 gram per 100 pounds of milk was found to be sufficient for the development of the characteristic flavor in less than five months. The control cheese did not develop the same intensity of flavor until ripened for more than one year. Smaller amounts of the enzyme preparation were proportionately less effective. The flavor and appearance of the "enzyme" cheese was characteristically different from that of the normal cheese.

Stärkle (7) has expressed the opinion that the formation of methyl ketones by the breakdown of certain fatty acids is responsible for the characteristic aroma of mold-ripened cheese. Hammer and Bryant (8) in a later study have concluded that methyl-n-amyl ketone is an important constituent of Blue cheese and that the compound is apparently formed from caprylic acid through the action of *Penicillium roqueforti* growing under conditions unfavorable for the normal growth of the mold. That flavor of Blue cheese which may be attributed to the methyl ketones appeared to be very pronounced in the cheese to which steapsin was added. Lane and Hammer (2) have observed that Blue cheese made from homogenized milk has a whiter appearance and that the mold has a more brilliant green color than the cheese made from unhomogenized milk. A similar difference between the "enzyme" and normal cheese was observed in this work, especially during the early stages of ripening. The "enzyme" cheeses could be identified solely by appearance owing to their chalky-whiteness and the brilliant green coloration of the mold. It has been noted at this station that the mold in Blue cheese made in the normal way loses much of its green color shortly after the cheese is placed in tin-foil. The green color of the mold reappears, however, when the cheeses approach the desired degree of ripeness. This loss and reappearance of color of the mold was never observed in the "enzyme" cheese.

Cheese to which steapsin was added was marketed through the same channels as other cheese made at this station. It was accepted by the trade as evidenced by repeat sales and by the fact that very few comments were received concerning any difference in the cheese. Some persons commented that the flavor was "too strong." A slight bitterness was always detected by those more familiar with the cheese, resulting in a distinct preference on the part of these individuals for cheese made in the normal way.

Data showing the relative rates of fat hydrolysis and protein degradation in "enzyme" and normal cheeses are shown in Table 1. The cheeses selected for these analyses were from batches made in the same plant, from the same milk supply, on succeeding days. The volatile acids were determined according to the method described by Currie (1). The amino nitrogen determinations were made using the Van Slyke microgasometric method. Results for the volatile acids are expressed as milliliters of N/10 acid per 100 grams of cheese, and for amino nitrogen as milligrams of nitrogen per gram of fat and moisture-free cheese.

TABLE 1

Fat hydrolysis and protein degradation of 4.5 month old cheese from normal milk and from milk to which steapsin was added

Treatment of milk	Volatile acids		Amino nitrogen
	Soluble	Insoluble	
	ml.	*ml.*	*mg.*
Normal	26.8	2.4	17.3
Steapsin	54.6	13.8	13.8

It is clearly shown that fat hydrolysis proceeded further in the cheese to which steapsin was added. The extent of protein degradation as indicated by the quantity of amino nitrogen in the cheese was probably not significantly different.

Several batches of cheese were made from pasteurized milk to which steapsin was added at the rate of two grams per 100 pounds of milk, but which were not inoculated with mold. These cheeses were handled in exactly the same way as the normal cheese except that they were not pierced. These cheeses had an intense rancid flavor when first examined after about one month. The rank flavor of butyric acid persisted to the virtual exclusion of all other flavors even up to the time the cheeses were over fifteen months old. The extent of fat hydrolysis in such cheese was as great or greater than in normal cheese or "steapsin" cheese inoculated with mold. Data on the soluble and insoluble volatile acids in these cheeses are shown in Table 2. Some idea of the volatile acids present may be gained by deter-

TABLE 2

Volatile acids in mold-free "steapsin" cheese

Lot no.	Age of cheese in months	Volatile acids in ml. N/10 acid per 100 gr. cheese		Barium value*
		Soluble	Insoluble	
1	8	80.1	8.0	
	12	111.5	12.7	41.54
2	3	72.6	2.7	
	12	96.8	11.1	41.06
3	7	97.2	16.8	
	11	96.8	13.85	41.38
4	7	105.2	16.0	
	11	107.9	16.1	41.37

* Determinations made by James Duncan, Assistant in Dairy Products, University of Minnesota.

mining the percentage of barium in the barium salt prepared from the volatile acids obtained on distillation, using the method described by Hammer and Sherwood (9). Barium values for the volatile acids obtained on distillation from mold-free "steapsin" cheese ranged from 41.06 to 41.54. These data also are shown in Table 2. Lane and Hammer (2) obtained barium values ranging from 40.42 to 42.00 for the volatile acids from normal Blue cheese 37 weeks of age. These results indicate that differences in the flavor of normal and mold-free "steapsin" cheese can not be ascribed to differences in the volatile acids in the cheese. The addition of steapsin accelerates the formation of the flavor constituents in the cheese but can not produce these flavors in the absence of the mold. These results are similar to those of Lane and Hammer (2) who found that in mold-free, unskewered cheese made from homogenized milk the flavor and aroma of methyl-n-amyl ketone could not be noted at any period during ripening.

SUMMARY

The ripening of Blue cheese may be accelerated by the addition of steapsin to the milk or to the curd. Approximately the same degree of flavor development was secured in five months in cheese to which commercial steapsin (Pfanstiehl) was added as in normal cheese in twelve months. While the "steapsin" cheese was accepted by the trade, competent judges considered it inferior to the normal cheese due to a characteristic bitter flavor.

Cheeses made with steapsin but which were not inoculated with mold became quite rancid and developed none of the typical flavor of Blue cheese. Although steapsin may accelerate the ripening of Blue cheese, it cannot produce the characteristic flavor of the cheese, unless *Penicillium roqueforti* is present and active.

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PRELIMINARY OBSERVATIONS ON THE TREATMENT OF PARCHMENT PAPER WITH SODIUM OR CALCIUM PROPIONATE[1]

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The protection of butter against surface molding has been an age-old problem. Many suggestions have been advanced as to methods for treating parchment wrappers and liners to destroy molds present on the paper or to inhibit subsequent growth of mold on the surface of butter or parchment. Numerous chemical compounds have been proposed and used but nearly all of them have had their disadvantages or have proved to be ineffective.[2]

Recently, sodium propionate and calcium propionate have been suggested as substances which might be effective inhibitors of mold growth when used for the treatment of parchment wrappers or liners. Consequently, preliminary trials have been run in our laboratories to determine the usefulness of these compounds[3] in the dairy industry.

EXPERIMENTAL METHODS

In these preliminary experiments, one-pound prints of commercial salted and unsalted butter were obtained and cut into eight equal parts. These portions were individually wrapped with pieces of parchment paper 4″ × 5½″ in size. Moisture and salt determinations were made on all lots of butter.

TRIAL I

Thirty-six eighth-pound prints of unsalted butter were placed upon a table in the laboratory and exposed to contamination from the air for 10 minutes so that five surfaces of each print were continuously exposed. Previous to, and during the exposure and wrapping, an electric fan was operated at full speed on the floor in the vicinity of the table. After exposure, the butter was immediately wrapped with parchment paper which had been immersed in 8, 16, or 20 per cent solutions (eight prints for each solution) of sodium propionate for a period of two minutes with twelve controls (three sets of four each, one set for each solution) wrapped in parchment immersed in sterile water. All the samples were placed in a humidor in which the atmosphere could be maintained at a humidity of approxi-

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[2] "Parchment Paper as a Source of Mold Infection in Butter," H. Macy and G. M. Pulkrabek. Minnesota Agricultural Experiment Station Bulletin 242. 1928.

[3] Sodium and calcium propionates were furnished through the courtesy of the E. I. Du Pont de Nemours & Company.

mately 100 per cent. The humidor was placed in a refrigerator for storage at 50° F. The samples were examined at the end of 2, 4, 6, and 12 weeks.

It may be noted in Table 1 that marked inhibition of mold growth on the surface of the butter or parchment resulted from the use of sodium propionate in the various concentrations mentioned above. Visible mold did appear in two weeks time on the surface of the samples wrapped with parchment paper treated with an 8 per cent solution, although the amount of growth was very slight. No visible growth was observed at the end of two weeks on the prints wrapped with parchment treated with the 16 or 20 per cent solutions and in four cases none appeared even after four weeks of storage. The 16 and 20 per cent solutions still exhibited a restraining effect at the end of six weeks storage and at 12 weeks the molds had not developed as extensively as they had on the controls.

TABLE 1

Effect of treatment of parchment with aqueous solutions of sodium propionate on the growth of mold on unsalted and salted butter exposed to the air for 10 minutes before wrapping

Per cent of sodium propionate	Extent of growth at 50° F. after							
	2 weeks		4 weeks		6 weeks		12 weeks	
	Un-salted	Salted	Un-salted	Salted	Un-salted	Salted	Un-salted	Salted
8 ..	8+	8–	8++	8–	8+++	6– 1+	8+++	4– 3+
16	8–	8–	1– 7+	8–	7+	7–	7++	7–
20	8–	8–	3– 4+	8–	7+	8–	7++	8–
Controls*	12+++	12++	12++++	12++		12++		12++

Note:
– = No visible growth.
+ = Scanty growth.
++ = Moderate growth.
+++ = Abundant growth.
++++ = Luxurious growth.

* Four controls for each lot of treated samples.

In the above and subsequent tables, the numbers followed by symbols may be interpreted for example as follows: 8+ means that all eight of the samples showed scanty growth; 1– 7+ means one sample showed no visible growth while seven showed scanty growth, etc.

Where less than eight samples are noted at any period, the remainder had been removed for analysis.

TRIAL II

In Trial II salted butter was subjected to the same conditions as described in Trial I. The moisture and salt in the butter were 15 per cent and 1.83 per cent respectively. The results of this trial are shown in Table 1. Complete inhibition of visible surface mold growth for a period of 12 weeks resulted when the parchments were treated with 16 per cent or 20 per cent solutions of sodium propionate. It is evident that the lower concentrations of sodium propionate were much more effective with the salted butter than with the unsalted. Only one of the eight samples wrapped with parchment treated with an 8 per cent solution showed any definite mold growth at the end of the 6-weeks period, one colony being observed on that print, but at the end of 12 weeks only four of the remaining samples were free from visible mold.

In this trial, the controls showed moderate growth and appeared quite different from the unsalted controls. The mold colonies seemed to be quite numerous but were very small, about two millimeters in diameter. In contrast the unsalted controls in Trial I showed large, spreading colonies.

TRIAL III

In this experiment, thirty-six eighth-pound prints of unsalted butter were heavily seeded with a suspension of mold spores. The mold cultures were obtained from samples of moldy butter brought to the laboratory from a commercial creamery. The spores taken from plate cultures of these molds were dispersed in sterile water in an atomizer and then sprayed over the surface of the prints of butter. The samples were thereafter wrapped in untreated and treated parchment as described in Trial I.

The results are presented in Table 2. Visible surface mold was observed after two weeks on samples wrapped in parchment treated with 8 and 16 per cent solutions of sodium propionate but some inhibition was noted in comparison with the controls. These prints were discarded at the end of the 4 weeks observation period because of the heavy mold growth. Complete inhibition of visible surface molding for a period of about three weeks resulted when the 20 per cent sodium propionate solution was used for treating the wrappers. This protection was not effective thereafter.

TABLE 2

Effect of treatment of parchment with aqueous solutions of sodium propionate on the growth of mold on unsalted and salted butter sprayed with a mold spore suspension before wrapping

Per cent of sodium propionate	Extent of growth at 50° F. after							
	2 weeks		4 weeks		6 weeks		12 weeks	
	Un-Salted	Salted	Un-salted	Salted	Un-salted	Salted	Un-salted	Salted
8	8+++	8++	8+++	7++		6++		6++
16	8++	7– 1+	8+++	3– 4+		3– 3+		3– 3+
20	8–	8–	8++	8–	8+++	6–		6–
Controls	12++++	12++++						

Note: On all samples indicated as + growth, only one visible colony was observed.

All controls showed a luxurious growth of surface mold and were discarded at the end of two weeks.

TRIAL IV

In this trial, salted butter was subjected to the same conditions as those outlined in Trial III. The butter was from the same lot as that used in Trial II. As shown in Table 2, an 8 per cent sodium propionate solution was not as effective as 16 or 20 per cent solutions in protecting the butter or wrappers from molding.

Complete inhibition of visible surface mold was evident on the prints wrapped with parchment treated with a 20 per cent solution of sodium propionate, even after twelve weeks of storage at 50° F. The controls in all cases were covered with a luxurious growth of mold at the end of two weeks. The mold colonies were small but nevertheless were just as objectionable as the larger colonies observed on unsalted butter.

TRIAL V

Trial V, in general, duplicated Trial I except that calcium propionate was used instead of sodium propionate and the butter was exposed to the air for a period of thirty minutes rather than ten minutes. The results are shown in Table 3. Mold growth was inhibited for a period of two weeks in all cases but at the end of four weeks all samples were moldy except two prints wrapped in parchment which had been treated with a 20 per cent solution of calcium propionate. After six weeks, all samples showed mold, but none were as bad as the controls. The highest concentration of propionate was clearly most effective.

TABLE 3

Effect of treatment of parchment with aqueous solutions of calcium propionate on the growth of mold on unsalted and salted butter exposed to the air for 30 minutes before wrapping

Per cent of calcium propionate	Extent of growth at 50° F. after							
	2 weeks		4 weeks		6 weeks		11 weeks	
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted
8	8–	8–	7++	7+	7++	7+	7+++	7+
16	8–	8–	7+	1– 6+	7++	2– 5+	7+++	2– 5+
20	8–	8–	2– 5+	7–	7+	7–	7++	6– 1+
Controls	12+++	12++	12+++	12+++	12+++	12+++	12++++	12++++

In Trial VI salted butter was used and subjected to the same conditions as those described in Trial V. The moisture and salt in this butter were 16.3 per cent and 1.75 per cent respectively. The results of this trial are shown in Table 3. It would appear that in the case of the prints wrapped in the parchment treated with 8 per cent and 16 per cent solutions of calcium propionate, less inhibition resulted than when sodium propionate was used. (See Table 1.) However, the period of exposure and the salt concentration of the butter used in this trial were more apt to favor mold growth. The controls in Trial VI showed abundant and luxurious growth whereas those in Trial II at no time during the 12 weeks showed more than moderate growth. Regardless of the differences in the composition and treatment of the butter, the results indicate a very marked inhibition when parchment treated with calcium propionate is used for salted butter.

TRIAL VII

In this experiment, conditions used in Trial III were duplicated. The results are shown in Table 4.

TABLE 4

Effect of treatment of parchment with aqueous solutions of calcium propionate on the growth of mold on unsalted and salted butter sprayed with a mold spore suspension before wrapping

Per cent of calcium propionate	Extent of growth at 50° F. after							
	2 weeks		4 weeks		6 weeks		11 weeks	
	Un-salted	Salted	Un-salted	Salted	Un-salted	Salted	Un-salted	Salted
8	8++	8–	8++	4– 4+	8++	8+	8+++	8++
16	8–	8–	4– 4+	8–	3– 5+	8–	3– 5+	7–
20	8–	8–	4– 4++	8–	4– 4++	8–	4– 4++	8–
Controls	12++++	12+++	12++++	12+++	12++++	12+++	12++++	12++++

A very marked inhibition of surface mold growth was observed on the prints wrapped with parchment treated with each of the three solutions. The samples wrapped in parchment treated with 16 and 20 per cent solutions showed no visible mold growth at the end of two weeks, and after four weeks one-half of the samples still showed no visible growth. In the case of samples wrapped in parchment treated with a 20 per cent calcium propionate solution, one-half of the samples remained free of visible mold growth during eleven-weeks storage. The controls in contrast showed luxuriant surface growth.

In comparing Table 4 with Table 2, a rather marked difference can be observed in the relative inhibitory power of sodium and calcium propionate.

Representative samples were picked from this trial and photographs were taken to bring out more clearly the difference in appearance between the controls and the prints wrapped in treated parchment. Figure 1 shows (a) a control, (b) a print wrapped in parchment treated with a 16 per cent solution and (c) a print wrapped in parchment treated with a 20 per cent solution of calcium propionate. At the time this picture was taken the butter had been stored for a period of 13 weeks.

TRIAL VIII

Conditions in Trial VIII were the same as those in Trial VII except that salted butter was used. This butter was from the same lot as that used in

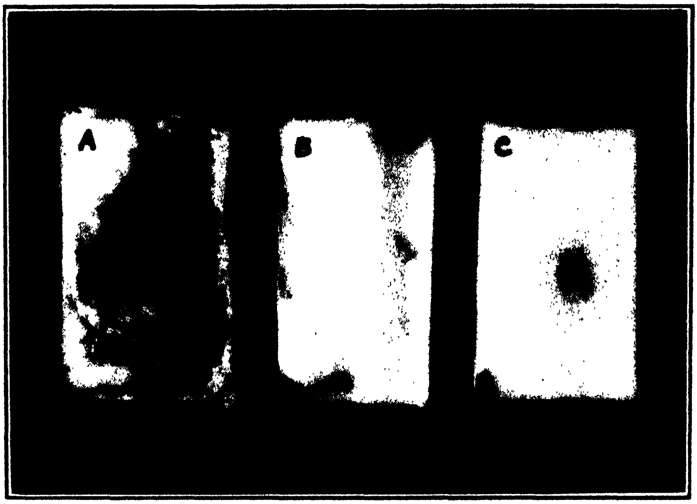

FIG. 1. This photograph shows the appearance of representative prints from Trial VII; A, a control, B, wrapper treated with 16 per cent solution, and C, wrapper treated with 20 per cent solution of calcium propionate. Thirteen weeks storage.

Trial III. Table 4 gives the results and again indicates the remarkable inhibitory power of calcium propionate.

Figure 2 shows three representative prints, (a) a control, (b) a print

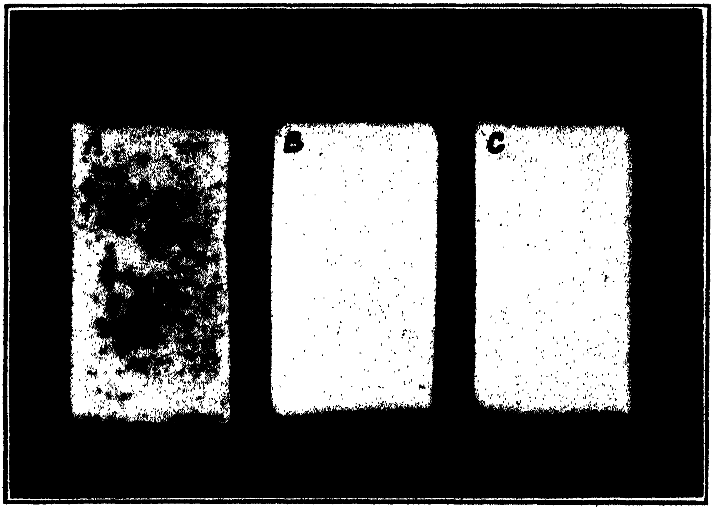

FIG. 2. This photograph shows the appearance of representative prints from Trial VIII; A, a control, B, wrapper treated with 16 per cent solution, and C, wrapper treated with 20 per cent solution of calcium propionate.

wrapped with parchment treated in a 16 per cent solution and (c) a print wrapped with parchment treated in a 20 per cent solution. Complete inhibition of visible surface mold throughout the duration of the storage period took place on samples wrapped with parchment treated with 16 and 20 per cent solutions of calcium propionate.

The controls as usual were covered with a luxuriant mold growth.

GENERAL DISCUSSION

In addition to the experiments previously described a number of other trials have been made using a mixture of sodium chloride and sodium propionate or calcium propionate. The results of these trials indicate that there might be some advantage in using a mixed solution of that sort for the treatment of parchment paper. In wrapping highly-salted butter, it may be advantageous to use a mixture of sodium chloride and one of the propionates for inhibiting molds as well as to prevent surface mottling.

In order to determine the effectiveness of the various concentrations of the propionates when the wrappers were merely dipped rather than soaked in the solutions, a duplication of trials I, II, V and VI was made. The results were fairly comparable with those obtained by immersing the parchment for two minutes except that they were more variable.

The ability of the sodium and calcium propionate solutions to inhibit mold growth when used in the treatment of butter wrappers is quite significant. The exact nature of the inhibitory principle has not as yet been determined.

When mold spots appeared on the surface of the butter the parchment paper was usually somewhat smudged where it was in contact with the moldy area. In most instances, the mold appeared to develop first on the butter.

In these preliminary trials, the butter and parchment were subjected to very extreme conditions regarding initial mold contamination and subsequent storage. The heavy seeding of the butter with mold spores from the air or by spraying with suspensions from cultures, was far more severe than that which butter would encounter in the ordinary manufacturing process or subsequent handling. Even under such conditions actual prevention of visible surface mold growth for a period of twelve weeks was observed in certain of the trials. Controls which were subjected to the same treatment except for the fact that they were wrapped with parchment immersed in sterile water, were covered with abundant growth in two weeks time.

During periods of observation it was noticed that the controls, particularly the unsalted butter, exhibited a decidedly cheesy flavor. This was not found to be true of prints wrapped with some of the treated parchments. Further studies on this point are also in progress.

The treated parchment did not appear to contribute any appreciable odor to the butter. Immediately after immersion the parchment dipped in calcium propionate solutions was quite bitter and the surface of the butter in contact with it was slightly bitter at first but soon lost such a taste. The sodium propionate solutions were somewhat sweet but did not seem to contribute any noticeable flavor to the surface of the butter.

Specific recommendations as to the proper concentrations of solutions to be used cannot be made at this time. Further trials are now in progress to determine the most practical and efficient methods which may be used for satisfactory inhibition of surface mold growth.

SUMMARY

1. Unsalted and salted butters deliberately contaminated with mold spores were wrapped in parchment papers, soaked either in sterile distilled water, or 8, 16 and 20 per cent solutions of sodium or calcium propionate, and stored for 11–12 weeks at high humidities at 50° F. to observe the extent of mold growth on the surface of the butter or parchment.

2. Marked inhibition of mold development occurred when the butters were wrapped in parchment papers treated with the solutions of sodium or calcium propionate, especially with the higher concentrations.

3. The inhibition was much more pronounced in the case of salted butter.

COMPARISONS OF THE INACTIVATION OF PHOSPHATASE ENZYME AND THE KILLING OF *ESCHERICHIA-AEROBACTER* ORGANISMS AS CRITERIA OF SATISFACTORY PASTEURIZATION OF ICE CREAM MIX[1]

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In a previous study (1) it was established that ice cream mix pasteurized at 150° F. (65.6° C.) may show a negative phosphatase test after an exposure of only 15 minutes, and in no case was a positive test observed after a 25 minute exposure. Mix held at 160° F. (71.1° C.) for 5 minutes invariably was negative to the phosphatase test.

The question arises whether mix pasteurized sufficiently to yield a negative phosphatase test is also adequately pasteurized with respect to the *Escherichia-Aerobacter* group of bacteria. Since both the phosphatase test and the determination of the presence of *Escherichia-Aerobacter* organisms are advocated as measures of the adequacy of pasteurization, the relationship between the two should be established. The protective action of the solids of the ice cream mix might not be the same for the enzyme and the organism.

The use of the two determinations already has been recommended for market milk (2, 3). A sample negative to the phosphatase test and containing organisms of the *Escherichia-Aerobacter* group is considered by many investigators to have been contaminated following satisfactory pasteurization. Such use of the two tests has proved of value to the writers in determining contamination of ice cream mix under plant conditions. However, the general applicability of the procedure to ice cream can be determined only by comparison of the two tests, using samples obtained under controlled conditions.

METHODS

The ice cream mix used was calculated to contain 12 per cent fat, 10 per cent serum solids, 15 per cent sugar, and 0.35 of one per cent gelatin. Ingredients used in preparing the mix included raw cream, raw skim milk, condensed skim milk, sugar and gelatin. Cultures of *Escherichia-Aerobacter* organisms grown in sterile milk for 24 hours at 37° C. and then held at 4° C. for 16 hours were added to all except the uninoculated control mixes in appropriate quantities immediately before pasteurization exposures were begun. The cultures used were the most heat-resistant of eighty-eight strains previ-

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[1] Contribution No. 176, Department of Bacteriology and No. 128, Department of Dairy Husbandry.

ously isolated from ice cream and were, with one exception, able to withstand 143.5° F. (61.9° C.) for 30 minutes when heated in ice cream mix of the composition used in these experiments. Laboratory pasteurization was made by carefully placing about 10 ml. of mix in the bottom of a sterile 18 × 150 mm. test tube which was closed by a sterile stopper and immersing the tube in a stirred water bath of the desired temperature to such a depth that the water reached at least two inches above the surface of the mix. In semi-commercial pasteurization the mix was heated in a 10 gallon steam-jacketed copper kettle equipped with a mechanical agitator, but with no provisions for the prevention of splattering or for insuring adequate heating of the foam. The samples were iced immediately after pasteurization. Phosphatase tests were made by the modified New York City field test devised by Scharer (4, 5) in all cases and also by the laboratory test of Gilcreas and Davis (6) in most cases. The numbers of *Escherichia-Aerobacter* organisms were determined by inoculations into triplicate tubes of brilliant green lactose peptone bile 2 per cent broth according to the tentative method outlined by the American Public Health Association (7). The most probable number of organisms per milliliter was determined from McCrady's tables of probability (8), with the exception of one series in which the numbers of *Escherichia-Aerobacter* organisms in the unpasteurized mixes were calculated from the microscopic counts of the organisms present in the cultures added.

EXPERIMENTAL RESULTS

The results of the first and second series of laboratory pasteurizations are shown in Tables 1 and 2, respectively. The *Escherichia-Aerobacter* organisms used did not survive in a sample of ice cream mix which was negative to the field or the laboratory test for the phosphatase enzyme, except in the case of the uninoculated control heated to 160° F. (71.1° C.) for 5 minutes in the second series (Table 2). Since only one of the triplicate tubes was positive in this case, the result was probably due to some error in technique. Except for this one instance, there was at least a slight residuum of active phosphatase present after all of the test organisms had been killed. In some cases a considerable amount of the enzyme remained active after death of the test organisms. A temperature of 145° F. (62.8° C.) for 30 minutes never inactivated all of the phosphatase and on some occasions failed to kill all of the *Escherichia-Aerobacter* organisms present in the mix. Exposure to 150° F. (65.6° C.) for 20 minutes destroyed all of the test organisms and gave a negative value for the New York City field test with but two exceptions, both in the first series. With but one exception, this same treatment reduced the phenol value of the samples, as measured with the Gilcreas and Davis test, to below 0.04 mg. of phenol per 0.5 ml. sample, the standard set by Gilcreas and Davis as the maximum permissible in a sample of market milk that

TABLE 1

Comparisons of phosphatase inactivation and destruction of Escherichia-Aerobacter organisms during laboratory pasteurizations of ice cream mix

Treatment of sample		Uninoculated control		Organism 107		Organism 11–1		Organism 116		Organism 15–1		Organism 32 C	
Temp. (°F.)	Time (min.)	Probable no. of *E.-A.* organisms per ml.[1]	Phos. test[2]	Probable no. of *E.-A.* organisms per ml.	Phos. test	Probable no. of *E.-A.* organisms per ml.	Phos. test	Probable no. of *E.-A.* organisms per ml.	Phos. test	Probable no. of *E.-A.* organisms per ml.	Phos. test	Probable no. of *E.-A.* organisms per ml.	Phos. test
Control	0	> 140	+++	> 14,000	+++	4,500	+++	11,000	+++	11,000	+++	4,500	+++
145	20	0	++	0.4	++	0	++	0	++	0	++	0.3	++
	30	0	+	1.4	+	0	+	0	+	0.3	+	0	+
150	10	0	+BA	0	+BA	0	+BA	0	+BA	0	+BA	0	+BA
	15	0	+BA	0	+BA	0	+BA	0	+BA	0	+BA	0	–
	20	0	–	0	–	0	±	0	–	0	±	0	–
	30	0	–	0	–	0	–	0	–	0	–	0	–
160	5	0	–	0	–	0	–	0	–	0	–	0	–
	10	0	–	0	–	0	–	0	–	0	–	0	–
	30	0	–	0	–	0	–	0	–	0	–	0	–

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[1] From McCrady's tables.

[2] New York City field test results (+BA = + only when butyl alcohol was used in reading test).

TABLE 2

Further comparisons of phosphatase inactivation and destruction of Escherichia-Aerobacter organisms during laboratory pasteurization of ice cream mix

Treatment of sample		Uninoculated control			Organism 60 gc			Organism 145			Organism 154			Organism 158 g2		
Temp. (°F.)	Time (min.)	Probable no. of *E.-A.* organisms per ml.[1]	Phos. test		Probable no. of *E.-A.* organisms per ml.	Phos. test		Probable no. of *E.-A.* organisms per ml.	Phos. test		Probable no. of *E.-A.* organisms per ml.	Phos. test		Probable no. of *E.-A.* organisms per ml.	Phos. test	
			NYC[2]	GD[3]		NYC	GD		NYC	GD		NYC	GD		NYC	GD
Control	0	> 14,000	+++	X	>140,000	+++	X	45,000	+++	> 0.15	45,000	+++	> 0.15	> 140,000	+++	> 0.15
145	20	0	++	X	0	++	X	0	++	> 0.15	0	++	> 0.15	0	++	> 0.15
	30	0	+	X	0	+	X	0	+	> 0.15	0	+	> 0.15	0	+	> 0.15
150	10	0	+BA	0.15	0	+BA	0.12	0	+BA	0.09	0	+	0.15	0	+	0.15
	15	0	+BA	0.05	0	+BA	0.05	0	+BA	0.04	0	+BA	0.09	0	+BA	0.09
	20	0	-	0.04	0	-	0.035	0	-	0.03	0	-	0.035	0	-	0.035
	30	0	-	0.025	0	-	0.015	0	-	0.02	0	-	0.02	0	-	0.015
160	5	0.4	-	0.02	0	-	0.015	0	-	0.015	0	-	0.01	0	-	0.03
	10	0	-	0.015	0	-	0.015	0	-	0.015	0	-	0.015	0	-	0.015
	30	0	-	0.015	0	-	0.01	0	-	0.01	0	-	0.01	0	-	0.01

[1] From McCrady's tables.

[2] New York City field test results (+BA = + only when butyl alcohol was used in reading test).

[3] Gilcreas and Davis test (phenol values).

X = no test made.

TABLE 3

Comparisons of phosphatase inactivation and destruction of Escherichia-Aerobacter organisms in ice cream mix pasteurized on a semi-commercial scale

Treatment of sample		Organism 11–1			Organism B-2-c			Organism 107			Organism 15–1			Organism 116		
Temp. (°F.)	Time (min.)	Probable no. *E.-A.* organisms per ml.[1]	Phosphatase test		Probable no. *E.-A.* organisms per ml.	Phosphatase test		Probable no. *E.-A.* organisms per ml.	Phosphatase test		Probable no. *E.-A.* organisms per ml.	Phosphatase test		Probable no. *E.-A.* organisms per ml.	Phosphatase test	
			NYC[2]	GD[3]		NYC	GD		NYC	GD		NYC	GD		NYC	GD
Control	0	18,000	+++	> 0.15	120,000	+++	> 0.15	56,000	+++	> 0.15	18,000	+++	> 0.15	14,000	+++	> 0.15
145	0	> 1,400	+++	> 0.15	> 1,400	+++	> 0.15	> 1,400	+++	> 0.15	> 1,400	+++	> 0.15	450	+++	> 0.15
	20	0.4	+	> 0.15	0.4	+	> 0.15	1.5	+	> 0.15	2.5	+	> 0.15	2.5	+	> 0.15
	30	25.0	+	> 0.15	0.4	+	> 0.15	2.5	+	> 0.15	4.5	+	> 0.15	2.5	+	> 0.15
150	0	> 1,400	++	> 0.15	> 1,400	++	> 0.15	> 1,400	++	> 0.15	> 1,400	+++	> 0.15	450	+++	> 0.15
	10	4.5	–	0.035	0.7	+s[4]	0.14	2.5	+s	0.15	9.5	+s	0.09	0.4	+s	0.15
	15	0.9	–	0.02	2.5	–	0.04	2.5	–	0.06	2.5	–	0.05	2.0	+s	0.04
	20	0.9	–	0.015	2.0	–	0.035	9.5	–	0.04	9.5	–	0.03	3.0	–	0.015
	30	0	–	0.015	2.5	–	0.01	0.9	–	0.03	2.5	–	0.01	2.5	–	0.01
160	0	4.5	–	0.01	2.5	+	> 0.15	25.0	+	> 0.15	2.5	+	> 0.15	4.5	+	> 0.15
	5	0.4	–	0.01	2.5	–	0.01	4.5	–	0.03	2.5	–	0.01	9.5	–	0.01
	10	0.7	–	0.01	2.5	–	0.01	2.5	–	0.01	4.5	–	0.01	2.5	–	0.01
	30	0.4	–	0.01	0.9	–	0.01	2.5	–	0.01	2.5	–	0.01	2.5	–	0.01

[1] From McCrady's tables.

[2] New York City field test (no butyl alcohol used).

[3] Gilcreas and Davis test (phenol values).

[4] +s = slightly positive.

had been adequately pasteurized. This value has been used arbitrarily in evaluating the results obtained in this study. At 160° F. (71.1° C.), 5 minutes exposure was sufficient to inactivate the phosphatase in all cases and to kill all of the test organisms, except in the one case previously mentioned.

The results of pasteurizations on a semi-commercial scale are given in Table 3. Although the results of the inactivation of phosphatase, as judged by the Gilcreas and Davis test, are in close agreement with those obtained in the laboratory pasteurization trials, the results obtained with the field test are not so satisfactory, probably due to the border-line cases which were missed because butyl alcohol was not used to concentrate the color. This series of tests was run before the butyl alcohol modification had been introduced. The presence of organisms of the *Escherichia-Aerobacter* group in all samples except one, irrespective of the heat treatment given, would indicate that contamination of the mix was occurring during or immediately following pasteurization. This is further emphasized by comparison with the results of the laboratory pasteurizations in which the same organisms were used. Contamination could easily have occurred, since no provisions were made for heating the foam and for prevention of splattering and drying on the sides of the container. In most cases, the numbers of organisms found in the pasteurized mix could be attributed to contamination of the 60 pound batch by as little as approximately one milliliter of the original heavily inoculated mix. Such a source is indicated by the lack of correlation between time of holding in each temperature series and the number of organisms surviving. The addition of such small quantities of incompletely pasteurized mix (approximately 0.04 of one per cent of the total volume) would be too small to be detected by any of the phosphatase tests now being used.

DISCUSSION

The results would indicate that in ice cream mix, the milk enzyme phosphatase has greater resistance to heat than have the more heat-resistant members of the *Escherichia-Aerobacter* group of organisms. More resistant strains than those used in these studies might possibly change the observed difference between enzyme inactivation and death of the organisms, but such strains do not occur commonly. Therefore, if organisms of this group are present in a sample negative to the phosphatase test, contamination is probable. That such contamination may occur during the pasteurization procedure is indicated by the results obtained in pasteurization on a semi-commercial scale as compared with laboratory pasteurization under controlled conditions.

The ease with which these two determinations can be made and the definiteness of the results obtained with each make them ideal for use in dairy control work, both in the plant and in the public health laboratory. By using the two determinations together, the diagnostic value of each is increased considerably. The difficulty of keeping ice cream equipment, particularly the viscolizer or homogenizer, in proper sanitary condition makes a reliable means of demonstrating contamination of considerable value.

CONCLUSIONS

1. Using ice cream mix as the substratum, the milk enzyme phosphatase is more resistant to heat than are the more heat-resistant bacteria of the *Escherichia-Aerobacter* group.

2. The presence of bacteria of the *Escherichia-Aerobacter* group in ice cream mix or ice cream which is negative to the phosphatase test can be considered an indication of contamination during or following pasteurization.

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STUDY OF PHYSICAL AND CHEMICAL PROPERTIES OF THE ADSORPTION "MEMBRANE" AROUND THE FAT GLOBULES IN CREAM. I. THE EFFECT OF THE ADSORPTION "MEMBRANE" OF SYNTHETIC CREAMS ON CURD TENSION OF COW'S MILK*

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The presence of an adsorption "membrane" around the fat globules in cow's milk has been demonstrated by the work of Storch (1), Völtz (2), Palmer and Samuelsson (3), Hattori (4), Palmer and Wiese (5), Schwarz and Fischer (6), and others. Palmer and Wiese (5), Wiese and Palmer (7), Rimpila and Palmer (8), have shown beyond any doubt that the adsorption layer consists of protein, phospholipides and other lipide substances. These investigations have proved that the protein of the "membrane" is different from any of the previously known proteins of milk, which is in agreement with the work of Hattori (4) and has been substantiated by Schwarz and Fischer (6).

While a considerable amount of work has been done on the methods of isolation of the "membrane" material, on its composition and the chemical nature of its constituents, much remains to be learned regarding its properties in relation to milk and milk products.

Palmer and Tarassuk (9) have shown that the "membrane" when released in buttermilk by churning sweet cream, lowers the curd tension. They have also shown that the artificial fat globule adsorption "membrane" derived by emulsifying butter fat in normal rennet whey likewise reduces the curd tension of milk plasma when released by churning but the adsorption "membrane" derived from skim milk does not possess this curd tension reducing property. Since the adsorption "membrane" derived from whey is very low in phospholipide content, as shown by Rimpila and Palmer (8), Palmer and Tarassuk suggested that the protein component of the fat globule "membrane" is the major factor concerned in the curd tension reducing effect of the "membrane."

The present study deals with the elucidation of the question as to which constituent, or constituents, of the adsorption "membrane"—the phospholipide, protein, or phospholipide-protein complex—is, or are, involved in the

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reduction of curd tension, as well as with the exposition of some other changes in the properties of buttermilks which were obtained from various synthetic creams whose fat globules adsorption "membrane" was derived from the material of known and predetermined chemical composition.

EXPERIMENTAL

General procedure and methods employed. Synthetic creams were made by emulsifying[1] fresh, pure, melted butter fat in an aqueous sol of the desired emulsifier to give the degree of dispersion of fat globules similar to that of natural cream, and to contain 27–30 per cent fat. The temperature of emulsification was 37° C. The cream emulsion was allowed to stand for two to three hours in ice water, observed for stability and warmed again to 37° C. at which time a sufficient amount of fresh raw skim milk was added to make 3.5–4.0 per cent "remade" whole milk. "Remade" whole was then separated to give "remade" cream and "remade" skim. "Remade" cream on standing (usually overnight) at 8°–10° C. was churned in a glass Dazey churn to give "remade" buttermilk.

Curd tension was determined by a modification of Hill's[2] method as described by Morris and Richardson (10), the only difference being that the temperature used was 35° C. instead of 37° C. Generally, rennet extract was used as a coagulant, but in some experiments, for definite purposes, other coagulants, such as rennet + $CaCl_2$, pepsin, pepsin + $CaCl_2$, and papain, were used.

Surface tension was determined at 20°–21° C. by means of the Cenco-du-Noüy tensiometer.

Casein, albumin, and globulin nitrogen was determined by Moir's (11) method, and total nitrogen either by the usual macro-Kjeldahl-Arnold method or by Cavett's (12) improved micro-Kjeldahl method.

Calcium and phosphorus were determined by the method of Morris, Nelson and Palmer (13). Butter fat was determined by the Babcock procedure using the Minnesota reagent except when otherwise indicated. Total solids were determined by the Mojonnier method.

Hydrogen ion concentrations were determined electrometrically using the Bailey hydrogen electrode.

Special experimental procedures will be described in connection with the several experiments.

EXPERIMENTAL RESULTS

Palmer and Tarassuk (9) have shown that when pure butter fat is emulsified in fresh rennet whey, the fat globule adsorption "membrane" so formed may have a greater reducing effect on the curd tension of buttermilk than the natural "membrane." This suggested the possibility of employing sols made from dried whey for the emulsification of butter fat in order to find out the nature of the adsorbed substance responsible for the lowering of curd tension.

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[1] The material was passed four times through a two-quart two-cylinder Club Aluminum Products Co. hand "homogenizer."

[2] The method is essentially the standard Hill test except for the older curd knives (diam. 2 inch) and the substitution of one ml. of rennet extract as coagulant for pepsin + $CaCl_2$.

The effect of adsorption "membrane" derived from dried whey on curd tension of buttermilk. Two kinds of dried whey were used as the source of the adsorption "membrane," dried acid whey and dried rennet whey.[3] Since the results were identical in both cases we shall present only the data on dried rennet whey cream and "remade" milks derived from it.

The dried whey had the following composition:

Lactose	75.58%	Ca	0.54%
Protein	14.50	P	0.52
Ash	6.80	H_2O	2.32
Fat	0.80		

The dried whey dispersed readily in water. An aqueous sol of the powder having the same or slightly higher concentration of total solids than in normal rennet whey was used as the emulsifying agent for pure, dry butter fat. The pH of this sol in experiment (A) was 6.45 and in experiment (C)—6.35. In experiments (A) and (C) mixed herd skim milk was used to make "remade" whole milk and in experiment (B) skim milk high in total solids (Guernsey milk) was used for the same purpose.

In their stability and microscopic appearance the whey-powder creams resembled very closely natural cream. There was no separation of phases even on standing for three days.

As can be seen from Table 1, the curd tension values of "remade" buttermilk in all experiments are strikingly low as compared with the respective "remade" whole and "remade" skim milks.

It is well known that a high positive correlation exists between the percentage of solids-not-fat of milk and its curd tension. Tarassuk and Richardson (14) reported a correlation coefficient of + 0.85 ± 0.3, calculated from the data on fifty samples of milk from different cows. The amount of casein seems to be the most important solids-not-fat component determining the curd tension of milk (14) (15). Espe and Dye (16), Doan and Welch (17), and many others, have reported that the curd tension of normal milk is almost a linear function of the casein content.

The "remade" milks in Table 1 represent a wide variation in percentage of solids-not-fat and the relationship between the solids-not-fat and the curd tension holds very well in case of all "remade" milks with the exception of the "remade" buttermilks. It is true that the percentage of casein in the "remade" buttermilk is slightly lower than in the "remade" skim milks but this fact cannot account for the extremely low curd tension of the "remade" buttermilks. Thus, in (A), the difference between the percentage casein in the "remade" skim and the "remade" buttermilk is 0.14, yet the curd tensions are respectively 50 and zero. The "remade" buttermilk in this case gave only a slightly visible coagulation and did not clot. There was no syneresis (separation of whey).

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[3] The dried wheys were experimental spray process products furnished by Abbotts Dairies, Inc., Cameron, Wisconsin.

TABLE 1

Curd tension and composition of various milks involving the use of synthetic cream made by emulsifying butter fat in dried rennet whey sol

Product tested	Curd tension	pH	Fat	Total solids	Solids-not-fat	Protein analysis[1]		
						Casein	Albumin-globulin	Non-prot. N
	gms.		%	%	%	%	%	%
Cream (A):								
Original skim	67		0.04	9.20	9.16	2.36	0.69	0.023
"Remade" whole	38		3.90					
"Remade" skim	50	6.57	0.40	9.10	8.70	2.06	0.69	0.041
"Remade" buttermilk	0	6.12	2.75	10.68	7.93	1.92	0.43	0.045
Cream (B):								
Original skim	145	6.51	0.04	10.64	10.60			
"Remade" whole	112	6.33	3.00	13.80	10.80			
"Remade" skim	121	6.44	0.08	10.70	10.62	3.64	0.59	0.005
"Remade" buttermilk[2]	48	5.78	1.25	12.22	11.97	3.52	0.66	0.005
Cream (C):								
Original skim	75	6.60						
"Remade" skim	60	6.60	0.11	9.28	9.17	2.44		
"Remade" whole	45	6.48	3.60	12.66	9.06	2.38		
"Remade" buttermilk	7	6.12	2.20	10.80	8.60	1.99		

[1] Percentage of casein, albumin and globulin was calculated by multiplying the percentage of nitrogen of the respective proteins by the factor 6.35. Non-protein N was determined by subtracting casein N and albumin-globulin N from total N.

[2] Curd tension of natural buttermilk obtained from the same whole milk as the skim and fat used in (B)—104.

A portion of the same "remade" buttermilk was pervaporated at room temperature to contain 22.84 per cent of total solids, 6.50 per cent of butter fat and 16.34 per cent of solids-not-fat. Normally, a milk with ten per cent solids-not-fat, or higher, has a curd tension above 100 grams (see Table 1 (B)); yet, this "remade" buttermilk with 16.34 per cent of solids-not-fat gave a curd tension of only 41 grams.

The data presented in Table 2 give additional and conclusive evidence of the futility of attempting to explain the low curd tension of the "remade" buttermilks by their content of solids-not-fat or of casein. It will also be noted from this table that the direct addition of an aqueous sol of dried whey to milk has no other effect on its curd tension except that of dilution.

TABLE 2

Curd tension and composition of "remade" buttermilk as compared to original skim and "remade" whole which have been diluted with water or aqueous sol of dried whey

Product tested	Curd tension	Fat	Total solids	Solids-not-fat	Casein
	gms.	%	%	%	%
Cream (A):					
Original skim	67	0.040	9.20	9.160	2.36
Original skim diluted 10% with H_2O	52	0.036	8.28	8.244	2.12
Original skim diluted 20% with H_2O	48	0.032	7.36	7.328	1.89
Original skim diluted 10% with aqueous whey powder sol	52	0.036	8.28	8.244	2.12
"Remade" buttermilk	*0*	*2.75*	*10.68*	*7.93*	*1.92*
Cream (C):					
"Remade" whole	45	3.60	12.66	9.06	2.38
"Remade" whole diluted 30% with H_2O	29	2.52	8.86	6.34	1.67
"Remade" buttermilk	*7*	*2.20*	*10.80*	*8.60*	*1.99*

The extremely low curd tension of these "remade" buttermilks becomes even more significant when we consider their pH. It can be seen from Table 1 that the "remade" buttermilks invariably had a much lower pH than their respective "remade" whole or skim milk. This fact will be treated in detail in the paper to follow; at the present it will suffice to point out that the curd tension of milk normally increases with drop in pH (14) (15). The peculiar relation of pH to curd tension of the "remade" buttermilks can be better appreciated from the fact that when the pH of the "remade" buttermilk of cream (B) (see Table 1) was increased from the initial 5.78 to 6.24 by adding 7 ml. of 0.2 N NaOH to 125 ml. of buttermilk, the curd tension was lowered from the initial 48 grams to 10 grams. At the same time the "remade" skim obtained from the same cream had a pH value of 6.44 and a curd tension of 121 grams, thus giving a difference of 111 grams between the curd tensions of "remade" skim and "remade" buttermilk.

A factor which could possibly account, at least partially, for the low curd tension of "remade" buttermilk is the degree of dispersion of butter fat in the buttermilk. Homogenization of whole milk reduces its curd tension, and it is generally believed that this is due to the effect of homogenization on the fat phase. Chambers (18), in his paper dealing with soft curd milk produced by intense sonic vibration, found, that, "the subdivision of as little as 0.25% of butter fat in a sample of skim milk brought about as great a reduction in tension as was accomplished by the vibrator on whole milk."

Microscopic examination of the "remade" buttermilk revealed that it contained a greater number of finely dispersed fat globules than its "remade" whole or "remade" skim milk. To determine the extent of the possible effect of the presence of these finely divided fat globules on the curd tension of "remade" buttermilk the following experiments were performed:

(a) *Partial removal of fat.* "Remade" buttermilk and "remade" skim from cream (B) (see Table 1) were warmed to 37° C. and centrifuged for 20 min. at 1800 r.p.m., using an International Equipment Co. size No. 2 centrifuge. The lower layer, poorer in fat, was pipetted out and the curd tension of the samples determined. The results are given in Table 3.

TABLE 3

Effect of partial removal of fat on curd tension of "remade" skim and "remade" buttermilk

Treatment of sample	Butter fat	Curd tension
	%	*gms.*
"Remade" skim (control)	0.08	121
"Remade" skim (centrifuged)	< 0.02	122
"Remade" buttermilk (control)	1.25	48
"Remade" buttermilk (centrifuged)	0.24	52

(b) *Addition of homogenized cream to skim milk.* Synthetic cream prepared from butter fat and sol of dried rennet whey, and natural cream, both containing 21.5 per cent of butter fat, were warmed to 110° F. and homogenized by using the single stage of a Gaulin homogenizer. The pressure applied was 2000 lbs. for the synthetic cream and 3000 lbs. for the natural cream.

In experiment I, a sufficient amount of each homogenized cream was added to the "remade" skim of cream (B) (see Table 1) and the curd

TABLE 4

Effect of degree of dispersion of butter fat on curd tension of milk

Exp.	Treatment of sample	Butter fat	Curd tension
		%	*gms.*
I	"Remade" skim	0.08	119
	"Remade" buttermilk	1.25	50
	90 ml. of "remade" skim + 10 ml. of 21.5% natural homogenized cream	> 2.0[1]	94
	90 ml. of "remade" skim + 10 ml. of 21.5% synthetic homogenized cream	> 2.0[1]	100
	Control: 90 ml. of "remade" skim + 10 ml. of natural non-homogenized cream	> 2.0[1]	100
II	Natural skim	0.04	74
	90 ml. of skim + 10 ml. of natural homogenized cream	> 2.0[1]	60
	90 ml. of skim + 10 ml. of synthetic homogenized cream	> 2.0[1]	64
	90 ml. of skim + 10 ml. of water	......	65

[1] Per cent of fat is estimated from the amount of cream added.

tension determined after a thorough distribution of the homogenized cream in the "remade" skim milk. The result was compared with the curd tension of the "remade" buttermilk from the same cream. In experiment II, the homogenized creams were added to natural skim milk. The data in Table 4 show that the presence of finely divided butter fat globules in the "remade" buttermilk can account for very little, if any, of its lost curd tension.

The effect of the adsorption "membrane" derived from the materials other than dried whey on the curd tension of buttermilk. Other materials used as the source of adsorption "membrane" for making synthetic creams were: skim milk powder, calcium caseinate, commercial gelatin, purified (isoelectric) gelatin, and tissue fibrinogen. The general procedure employed in making the synthetic creams and "remade" milks has been described above. In all cases the skim milk used for making "remade" whole milk from synthetic cream was fresh raw mixed herd skim milk. Other data pertinent to the synthetic creams and their "remade" milks are as follows:

(a) *Skim milk powder cream.* Spray process skim milk powder, easily dispersable in water, was used. An aqueous sol, containing 9 per cent total solids, served as the emulsifying agent. The fat emulsion appeared to be very stable and there was no separation on standing in ice water for 3.5 hours. The churning time of the "remade" cream was 50 minutes.

(b) *Calcium caseinate cream.* The emulsifying agent was a 3 per cent calcium caseinate sol.[4] The pH of the sol was 7.31. The cream emulsion was stable and the churning of the "remade" cream took 40 minutes.

(c) *Gelatin cream.* Several separate experiments were conducted using gelatin as the sole source of adsorption "membrane." In experiments I and II a commercial gelatin and in experiment III a purified (isoelectric) gelatin was used. The isoelectric gelatin contained 0.05 per cent ash and 16.03 per cent nitrogen. The isoelectric gelatin being pure protein removed any possibility that the impurities usually present in commercial gelatin could be of significance in the curd tension tests. In all cases the emulsifying agent was a 0.5 per cent gelatin sol. The pH of the commercial gelatin sol was 5.42 and that of the isoelectric gelatin was 4.63. The gelatin creams were very stable and there was no separation of phases on standing at 7° C. for as long as 27 hours. The churnability of the gelatin "remade" creams was quite similar to that of the calcium caseinate "remade" cream, the average churning time being from 40 to 45 minutes.

(d) *Tissue fibrinogen cream.* Tissue fibrinogen—a substance known to be a lecithoprotein (19)—was employed as emulsifying agent in several experiments in order to determine whether phospholipide-protein complex is important in the curd tension reduction of buttermilk. The experiments presented some unexpected problems regarding the stability and churnability of emulsions prepared by this agent, which first required solution. The starting material[5] was a concentrated paste and represented the globulin fraction which had been precipitated from a lung tissue extract by half saturation with $(NH_4)_2SO_4$. For making synthetic cream, a one per cent aqueous fibrinogen sol was employed. Since the fibrinogen paste contained a considerable amount of $(NH_4)_2SO_4$ the fibrinogen sol, prior to its use, was dialyzed for three hours against distilled water. At the end of this period the dialysate gave only a faint test for SO_4^{--}. Dialysis for a longer period caused the precipitation of globulin. The pH of the fibrinogen sol was 5.82. A preliminary experiment on the emulsification of butter fat in a fibrinogen sol at the pH of milk (6.64) showed that it was impossible to produce a normal cream emlusion. In view of this unexpected finding a study of the effect of the pH of the sol on the character of the resulting emulsion was undertaken with the following results:

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[4] The calcium caseinate used in this experiment was an experimental commercial product manufactured as follows: Casein, precipitated by HCl and washed twice with water, was made up to 7–8 per cent total solids with water. At this time $Ca(OH)_2$ was added until litmus paper turned blue; the calcium caseinate suspension was heated to 160° F. and homogenized at 3000 lbs. pressure. The homogenized product was dried by a spray process, giving a very light, fluffy powder, easily dispersed in water to give a milky sol.

1. *Emulsion with fibrinogen sol, pH = 5.82, or lower.* An unstable emulsion resulted, characterized by a pronounced clustering of fat globules, rapid rise of the cream and oiling off.
2. *Emulsion with fibrinogen sol, pH = 6.94.* The emulsion was even more unstable than in trial 1 of this series: it exhibited the same characteristics but to a greater extent.
3. *Emulsion with fibrinogen sol, pH = 7.30.* A fairly stable emulsion was obtained. The dispersion of fat globules was normal except for a small amount of clustering. There was no oiling off of the cream on standing.

The fibrinogen cream from which "remade" milks were made was, therefore, obtained by emulsifying butter fat in fibrinogen sol the pH of which was increased to 7.32 by adding NaOH. The separation of the "remade" whole appeared to be normal. The churning of the cream from the separated "remade" whole milk was characterized by the absence of foam and a relatively short churning time. The cream churned in less than 30 minutes.

The curd tension of the "remade" buttermilks from calcium caseinate and gelatin creams was zero in all cases as can be seen from Table 5. In the case of the "remade" buttermilks from these creams, as well as from some of the dried whey creams (see Table 1), total absence of clotting resulted when rennet was used as coagulant. The "remade" buttermilk from gelatin cream (experiment II) stood for an hour at 35° C. after rennet had been added yet remained perfectly liquid without a sign of visible coagulation. In experiment II (gelatin cream), after the first milliliter of rennet had been

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[5] Fibrinogen was supplied by the Wm. S. Merrel Company, Manufacturing Pharmacists, Cincinnati, Ohio.

TABLE 5

Curd tension, pH, surface tension and composition of "remade" milks from skim milk powder, calcium caseinate and gelatin creams

Product tested	Curd tension	pH	Surface tension at 20–21° C.	Total solids	Fat	Solids-not-fat	Casein
	gms.		*Dynes per cm.*	%	%	%	%
(a) Skim milk powder cream:							
Original skim	84						
"Remade" whole	64				3.70		
"Remade" skim	74	6.42		10.03	0.26	9.77	
"Remade" buttermilk	49	5.88		10.45	0.30	10.15	
(b) Calcium caseinate cream:							
Original skim	82			9.52	0.02	9.50	
"Remade" whole	60				3.50		
"Remade" skim	69	6.60		9.94	0.06	8.88	
"Remade" buttermilk	*0*	6.21		9.08	0.62	8.46	
Original skim diluted 13% with 3% Ca-caseinate sol (control)	68						
"Remade" skim diluted 13% with 3% Ca-caseinate sol	58						
(c) Gelatin creams:							
Exp. I							
Original skim	78						
"Remade" skim	59	6.63		8.55	0.03	8.52	
"Remade" whole	56				3.10		
"Remade" buttermilk	*0*	6.09		9.12	1.40	7.72	
Original skim diluted 13% with 0.5% gelatin sol (control)	56						
Original skim diluted 13% with H_2O	58						
Exp. II							
Original skim	84				...	...	...
"Remade" skim	55	6.64	45.5	8.40	0.04	8.30	...
"Remade" whole	42	...	42.7		3.70	...	...
"Remade" buttermilk	*0*	6.09	33.6	8.72	1.40	7.32	...
Exp. III							
Original skim	73	6.64	47.6	...	...	...	...
"Remade" skim	48	6.64	46.9	8.28	0.02	8.26	2.30
"Remade" whole	46	6.52	43.6	...	3.60	...	...
"Remade" buttermilk	*0*	6.10	38.5	10.08	2.80	7.28	1.94
"Remade" skim diluted 15% with H_2O	30	...	...	7.05	0.017	7.02	1.95
(d) Tissue fibrinogen cream:							
Original skim	79	6.61	46.9	...	...	...	...
"Remade" skim	57	6.65	46.2	8.20	0.02	8.18	2.48
"Remade" whole	49	6.63	...	...	4.07	...	...
"Remade" buttermilk	15	6.76	42.7	6.45	0.95	5.50	1.54
"Remade" skim diluted 32% with H_2O[1]	17	...	...	5.58	0.013	5.56	1.69

[1] Diluted to the per cent of solids-not-fat of the "remade" buttermilk.

added to the "remade" buttermilk and it had stood for 10 minutes at 35° C., the addition of two more milliliters of rennet to the "remade" buttermilk produced a partial coagulation but no clotting, as there was no separation of whey on standing. The "remade" buttermilk from calcium caseinate cream failed to give a visible coagulation after 20 minutes standing at 35° C. even on the addition of 3 ml. of concentrated rennet extract per 100 ml. of buttermilk.

All other "remade" milks, on the addition of rennet in the amount used in the determination of curd tension value (1 ml. of concentrated rennet extract per 100 ml. of milk), clotted within 30 seconds, usually instantaneously.

It is evident from Table 5 that the phenomenon of non-coagulation with rennet is restricted only to the "remade" buttermilks, since the curd tension of the other "remade" milks, as well as the curd tension of natural (original) skim to which gelatin or calcium caseinate sol had been added directly, was normal except for the effect of dilution. Furthermore, the above phenomenon is permanent, at least within the life of the product, since the samples of the "remade" buttermilk, when tested after standing six, fourteen, twenty-four, and forty-eight hours, all failed to coagulate with rennet. It should be kept in mind that the fat globule adsorption "membrane" in the case of gelatin creams, and especially in experiment III in which isoelectric gelatin was used, must have been composed entirely of protein material.

In the case of "remade" milks from skim milk powder cream, a reduction in curd tension of the "remade" buttermilk is evident; however, it is by no means so profound as in the case of the "remade" buttermilk from dried whey, gelatin or calcium caseinate cream.

The data in Table 5 on "remade" milks from tissue fibrinogen cream, the cream whose fat globule adsorption "membrane" was derived exclusively from lecithoprotein-complex material, show conclusively that this adsorption "membrane" had no effect on the curd tension of buttermilk. A rather low curd tension of the "remade" buttermilk is explained entirely by its low content of solids-not-fat because, when the "remade" skim was diluted to contain the same percentage of solids-not-fat as in the "remade" buttermilk, it gave an equally low curd tension. The low percentage of solids-not-fat in the "remade" buttermilk most likely is due to the retention of some of the cream plasma solids in the bowl of the separator in the process of separation of the "remade" whole milk since the fibrinogen-butter fat emulsion did exhibit a greater tendency for producing clustering of butter fat globules than the other creams.

Another important fact brought out by the experiment with tissue fibrinogen cream is the significance and the relation of pH and surface tension to the phenomenon of low curd tension, or total noncoagulation of the "remade" buttermilk. As can be seen from Tables 1[6] and 2, in all cases in which the "remade" buttermilk had a low curd tension or did not coagulate with rennet, the pH and the surface tension of such buttermilk are significantly lower than the pH of other "remade" milks from the same cream. On the average, the pH was lower by from 0.5 to 0.6 units and the surface tension lower by about 7 dynes per cm. Surface tension of the tissue-fibrinogen "remade" buttermilk was only 3.5 dynes lower than that of the "remade" skim milk and the pH actually slightly higher.

The effect of addition of $CaCl_2$ on the curd tension of "remade" buttermilks. It is well known (20) that the addition of $CaCl_2$ to milk increases the rate of rennet coagulation, produces harder curd and speeds up the syneresis of the clot. This effect of $CaCl_2$ is due both to the lowering of the pH and to the increase of Ca^{++} in milk. The presence in milk of an adequate concentration of calcium ions is essential for a normal clotting with rennet.

The "remade" buttermilks which did not clot with rennet, clotted on the addition of $CaCl_2$ prior to the addition of rennet or on the addition of $CaCl_2$ in a form of pepsin + $CaCl_2$ (Hill's coagulant) or rennet + $CaCl_2$. In such cases a normal coagulation and wheying of clot took place, and the curd tension of the buttermilk was restored, at least partially, if not completely, in some instances. The magnitude of these curd tension values, as compared to the curd tension values of the corresponding other "remade" milks, is shown in Table 6.

It is interesting to note from the data in Table 6 that it is necessary to add to the "remade" buttermilks a rather large amount of $CaCl_2$ in order to obtain a clot. One ml. of 5 per cent $CaCl_2 \cdot 2H_2O$, which is equivalent to 13.6 mgms. of calcium added per 100 ml. of buttermilk, failed to produce a clot.

Apparently the explanation of the non-coagulation of the "remade" buttermilk in experiment II, gelatin group, Table 5, seems to be lack of Ca^{++}, yet, theoretically, there should have been more Ca^{++} in this "remade" buttermilk than in the other "remade" milks in the experiment since its pH was much lower. The problem may also be considered in relation to the Ca^{++} content of the products in the calcium caseinate experiment in which all the "remade" milks must have been actually higher in total calcium than the original skim milk. The most widely quoted mean value for the soluble calcium of milk (21) is 32 per cent of the total calcium, which would be about 30 to 40 mgms. of calcium per 100 ml. of milk. Only a portion of the soluble calcium exists in milk as Ca^{++} due to the occurrence of calcium citrate which is soluble but only slightly ionizable (20). Natural milk can be diluted with water to the extent of 40 per cent and, with a rennet concentration such as used in the

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[6] The surface tensions of "remade" milks from whey powder cream (C) were as follows: "Remade" skim—49.0 dynes per cm., "remade" whole—46.2 dynes per cm., and "remade" buttermilk—38.5 dynes per cm.

TABLE 6

Curd tension of "remade" buttermilk on the addition of $CaCl_2$ as compared to other "remade" milks

Product tested	Curd[1] tension	Remarks
	gms.	
Calcium caseinate cream:		
Coagulant: Pepsin + $CaCl_2$		
Original skim	121	
"Remade" skim	95	
"Remade" whole	80	
"Remade" buttermilk	89	
Gelatin cream:		
Coagulant: Pepsin + $CaCl_2$		
Exp. I		
"Remade" skim	70	
"Remade" buttermilk	65	
"Remade" whole	57	
Coagulant: Rennet		
Exp. II		
"Remade" skim	55	
"Remade" whole	42	
"Remade" buttermilk + 1 ml. of 5% $CaCl_2 \cdot 2H_2O$[2]	0	Only partial coagulation, resembling precipitation rather than clotting.
"Remade" buttermilk + 4 ml. of 5% $CaCl_2 \cdot 2H_2O$	41	
Exp. III		
"Remade" skim	48	
"Remade" skim diluted 15% (diluted to the % of S.N.F. of the "remade" buttermilk)	30	
"Remade" skim diluted 15% + 4 ml. of 5% $CaCl_2 \cdot 2H_2O$	62	
"Remade" buttermilk + 4 ml. of 5% $CaCl_2 \cdot 2H_2O$	45	
Dried whey cream:		
Coagulant: Rennet		
"Remade" skim	48	
"Remade" whole	41	
"Remade" buttermilk	1	
Coagulant: Pepsin + $CaCl_2$		
"Remade" skim	57	
"Remade" buttermilk	25	
"Remade" whole	46	

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[1] For curd tension values of "remade" milks from Ca-caseinate and gelatin creams, when rennet was used as a sole coagulant, see also Table 5.

[2] In all cases the stated amount of $CaCl_2$ added to 100 ml. of milk.

determination of curd tension, will still give a satisfactory clot. Of course, it must be appreciated that a decrease of ionizable calcium is not directly proportional to the degree of dilution because the dissociation would be greater in a more dilute solution.

Any discussion on the amount of Ca^{++} necessary to insure a normal clot with rennet is purely speculative since we have as yet no satisfactory means for measuring Ca^{++} in milk. However, on taking into consideration the history of the "remade" milks, we are justified in assuming that the cause of non-coagulation of the "remade" buttermilks is not a low concentration of Ca^{++}. Further evidence in support of this is seen in Table 7 which gives the Ca and P analysis of the whey from the "remade" buttermilk (gelatin cream, experiment II) in comparison with the whey from the "remade" skim. Although neither rennet nor pepsin would clot this "remade" buttermilk, it was found that papain, when used at proper concentration, would clot it with a satisfactory separation of clear whey. The optimum proteolytic activity of papain, according to Willstätter (22), lies at the isoelectric point of a protein. Kometiani (23), who used papain for obtaining whey from a heated milk, points out that the proteolytic activity of papain at the pH of milk is not strong enough to affect the calcium and phosphorus content of whey significantly.

TABLE 7

Calcium, phosphorus and ash contents of papain whey from "remade" buttermilk and "remade" skim milk of gelatin cream experiment

Exp.	Product	Milligrams per 100 gms. of whey		Ash
		Ca	P	
				%
II	Whey from "remade" buttermilk	36.80	45.40	0.404
	Whey from "remade" skim	29.98	40.02	0.450

The procedure for clotting milk with papain was similar to Kometiani's and, briefly, was as follows:

> To 100 ml. of milk, warmed to 60° C., 5 ml. of 2.5 per cent aqueous solution of papain powder were added and the milk allowed to stand at 60° C. for 20 minutes. The separated whey was filtered and the calcium and phosphorus determined in duplicate 25 ml. aliquots of the whey from each sample.

On the addition of papain the "remade" buttermilk clotted almost instantaneously and the "remade" skim clotted in about four minutes.

The calcium content of the whey from the "remade" buttermilk was actually higher than that of the whey from the "remade" skim milk; this is to be expected since the pH of the buttermilk was 6.09 as compared with 6.64 for the "remade" skim.

SUMMARY

Synthetic creams were made by emulsifying pure butter fat in the following aqueous sols: Dried whey sol, skim milk powder sol, calcium caseinate sol, gelatin sol, and tissue fibrinogen (lecithoprotein material) sol. The resulting synthetic creams were dispersed in normal milk plasma to obtain "remade" whole milks which were separated to give the corresponding "remade" skim milks and "remade" creams. The "remade" buttermilks were prepared by churning the "remade" creams.

The fat globule adsorption "membrane" from dried whey cream, when released in buttermilk by churning the "remade" cream, exerted a profound effect on the rennet clot of the buttermilk. This effect was characterized either by the total inhibition of clotting or by the formation of very soft and fragile curd with little or no syneresis.

A still more pronounced effect of the fat globule adsorption "membrane" on the rennet clot of "remade" buttermilk was found in the cases of gelatin cream and calcium caseinate cream. "Remade" buttermilk from either of these creams invariably had no curd tension whatever and did not show even a visible coagulation on the addition of rennet.

Although the addition of $CaCl_2$, in rather large concentration, to buttermilks from the above creams restored somewhat their curd tension values, it has been shown by calcium and phosphorus analysis of the papain clot whey from the buttermilk of gelatin cream that the ultimate cause of this phenomenon of low curd tension, or non-coagulation, is more deep seated than that of lack of Ca^{++}. This being the case the $CaCl_2$ cannot be regarded as restoring the clotting properties in these experiments simply because it supplies Ca^{++} to the system.

The fat globule adsorption "membrane" from skim milk powder cream had only a relatively slight curd tension reducing effect on its "remade" buttermilk as compared with the effect exerted by the adsorption "membrane" from dried whey cream.

The fat globule adsorption "membrane" derived from tissue fibrinogen (lecithoprotein complex material) had no effect on the curd tension of "remade" buttermilk.

The phenomenon of total non-coagulation with rennet, or abnormally low curd tension of "remade" buttermilks, was always accompanied by considerably lower pH and lower surface tension of the "remade" buttermilks as compared with the pH and surface tension of their respective whole and skim milks. The factors involved in the decreased pH and lowered surface tension and their relation to the loss of clotting ability have been the subject of extensive further study by us.[7]

It may be stated briefly at this time that the total destruction of the clot in the case of certain "remade" buttermilks was found to be due largely to the liberation of certain fat acids and their adsorption by the calcium caseinate, preventing the clotting of the milk by rennin. It was also found that curd tension is not seriously affected, if affected at all, by butter fat hydrolysis except when an unnatural emulsifying agent is substituted for the natural fat globule adsorption "membrane" of cow's milk. Furthermore, when lauric, myristic or palmitic acid is added directly to skim milk or allowed to develop as the result of hydrolysis of esters of these fat acids by "natural" esterase in the milk, the normal clotting properties of the milk are inhibited. Other important aspects of these findings will be presented in detail in the papers to follow.

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[7] Part of this further study was a post doctorate work and was done by the senior author in the Dairy Chemistry Laboratory of the Dairy Industry Division, University of California, Davis.

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INADEQUACY OF A WHOLE MILK RATION FOR DAIRY CALVES AS MANIFESTED IN CHANGES OF BLOOD COMPOSITION AND IN OTHER PHYSIOLOGICAL DISORDERS[1]

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Early quests have revealed that natural milk of the cow, though adequate in the early stages of the life of the calf, yields disastrous results if fed as the sole ration for any great length of time after the normal suckling period.

Numerous experiments have been conducted yielding much information relative to the deficiencies of whole milk as the sole diet for mammals. Since the extensive literature relative to the subject has been adequately reviewed and presented by Herman (15), no attempt is made to repeat or to condense the available facts. In the light of the results reported it was deemed advisable to extend the study of the various physiological changes manifested in calves restricted to milk rations. Special emphasis was placed on the changes in composition of the blood in an attempt to supplement and enlarge the reported symptomatology.

EXPERIMENTAL PROCEDURE

Selection of animals. Twelve calves as described in Table 1 were used in this experiment. Their dams were normal individuals receiving adequate rations consisting of grain and hay. The only requisites in the selection of the calves were good health and normal appearance. They were placed on experiment shortly after birth and retained until either death ensued or severe symptoms of malnutrition deemed supplementation of the ration advisable. In the latter case the calves were transferred to another experiment which will be reported in a later paper.

Feeding and management. All calves, with the exception of E–236, had continuous access to colostrum during the first two or three days after birth, the time they remained with their dams. Subsequently they were fed fresh raw milk by pail twice daily.

The milk was produced by the College Dairy Herd which consisted of Ayrshires, Jerseys, Guernseys and Holsteins, the last named predominating. The milking cows received medium quality alfalfa hay, corn silage, and a grain mixture consisting of 250 pounds of yellow corn, 250 pounds of barley,

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[2] The data in this paper are from a thesis presented by G. H. Wise in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, Minnesota Agricultural Experiment Station.

TABLE 1

Calves restricted to a whole milk diet

No. of animal	Breed	Sex	Age started	Age removed	Cause for discontinuance	Disposition
			days	*days*		
E–230	Grade Holstein	Female	3	103	Lethargy	Transferred
E–231	Grade Holstein	Female	3	196	Death	....
E–232	Grade Holstein	Female	3	273	Paresis	Transferred
E–233	Grade Holstein	Female	3	324	Stiffness	Transferred
E–234	Grade Holstein	Female	3	289	Anemia	Transferred
E–235	Grade Holstein	Female	4	98	Death	....
E–236	Grade Holstein	Male	6	270	Death	....
E–237	Grade Holstein	Female	3	55	Death	....
E–238	Purebred Guernsey	Male	8	161	Anemia	Transferred
E–239	Purebred Ayrshire	Male	3	181	Anemia	Transferred
E–240	Purebred Jersey	Male	2	97	Death	....
E–241	Purebred Holstein	Female	6	83	Tetany	Transferred

250 pounds of oats, 100 pounds of bran, 100 pounds of linseed meal, 50 pounds of cottonseed meal and 15 pounds of common salt. During the winter, the cows were confined to the barn, and during the remainder of the year, they were released into pasture which supplied not more than half the nutrient needs of the cows. The quantity of milk fed was regulated by the appetite of the calf and its subsequent response with respect to digestive disturbances.

Water generally was restricted. Only four, E–231, E–232, E–233 and E–234 received water, which was offered twice daily during summer and early fall. It was hoped that by withholding water the desire for milk would be increased. The validity of this assumption is doubtful. Commercial iodized sodium chloride was provided *ad lib*, but relatively small amounts were consumed.

The calves were confined in individual board-walled box stalls that were protected from direct sunlight. The concrete floors were kept well covered with wood shavings. The calves were not muzzled and consequently had free access to wood boards, shavings and hair. Since previous experiments (12) revealed that consumption of the foregoing materials does not vitiate the harmful effects of prolonged milk feeding, the calves were not muzzled.

Determination of various physiological changes. The recorded observations included thereon: (1) general health, (2) growth, (3) blood analysis and (4) post mortem examinations.

1. *General health.* A detailed record of the general appearance and the reactions of each calf throughout the experiment was made.

2. *Growth.* This was estimated by two common physical measurements, body weight and height at withers. Early morning weighings, before feeding, were made the first, tenth, twentieth, twenty-ninth and thirtieth days of the thirty-day experimental period. Height at the withers, the criterion of skeletal development, was determined the last day of the period.

3. *Blood analysis.* Blood was analyzed for corpuscular-plasma volume, hemoglobin and sugars. The plasma was analyzed for calcium, inorganic phosphorus, magnesium and fat. These analyses were made weekly. The anticipated advantages of this frequency were as follows: (1) permits each animal to serve as its own control, (2) establishes the time of change in any constituent, (3) detects the sequence of changes, and (4) reveals the duration of the change.

a. *System of preparing blood and plasma for analysis.* Samples of blood were taken from the jugular vein about three hours after the morning feeding. The blood was received into a 15 ml. centrifuge tube containing 0.15 ml. of saturated sodium citrate solution. Since the dilution was constant for all samples, this factor was not considered in any of the analytical results.

Portions of the citrated blood were withdrawn for hematocrit, for hemoglobin and for sugar determinations. The remainder was centrifuged to separate the plasma from the corpuscles.

In order to withdraw the maximum amount of plasma without disturbing the compacted cells, the plasma was removed by siphoning. (See Fig. 1.)

b. *Analytical procedures for blood and plasma.* The corpuscular-plasma volume was measured by the Wintrobe method (33, 34). According to this investigator the type of anti-coagulant plays a role in the volume of the individual corpuscles and, consequently, is an important factor that should be considered in the measurement of the total volume of packed cells. Green and Macaskill (13) contended that some plasma must always remain entangled in the midst of the compacted corpuscles regardless of the kind of anti-coagulant used. According to their calculations about one-third of the "red precipitated mass," designated as *volume of red cells,* is plasma. In view of the controversial status the volume per cent of cells presented herein are uncorrected for shrinkage of corpuscles.

Hemoglobin was determined according to the method of Newcomer (24). The filtrates for the blood sugar analysis were prepared by the procedure of Somogyi (30). The analyses of the filtrate were made according to the methods reported by Shaffer and Somogyi (25). The method employed for fat determination was that devised by Allen (1). The Clark-Collip (7) modification of the Kramer-Tisdall method was used for the determination of calcium of the plasma. The supernatant liquid from the precipitated calcium was preserved for magnesium determinations. The procedure followed in the magnesium analysis was that of Denis (10) with several adopted modifications (9). The phosphate of the precipitated magnesium ammonium phosphate was determined colorimetrically by the procedure of Bodansky (3). Inorganic phosphorus of the plasma was calculated from an analysis of the trichloracetic acid filtrate for phosphorus according to the method outlined by Bodansky (3). The major obstacle encountered in this procedure was an occasional turbidity of the filtrate. This was observed most frequently in

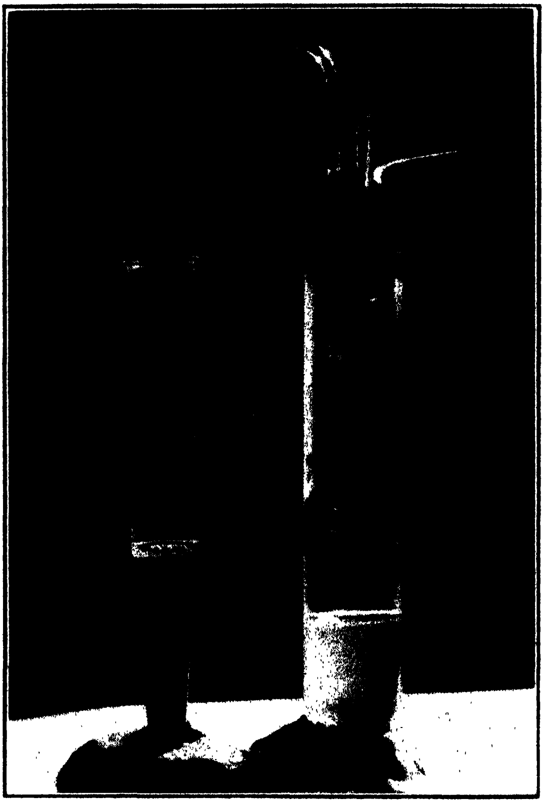

FIG. 1. Apparatus for removing plasma from centrifuged blood. The long leg of the siphon tube is extended through one of the openings of a three-hole rubber stopper. A short glass tube bent almost at right angles for connection to a suction pump and a straight short glass tube for vacuum control are inserted, respectively, into the remaining two openings. The stopper with its attachments is fitted tightly into the mouth of a test tube, the receptacle. By careful manipulation with this apparatus the plasma may be removed conveniently within 0.3 mm. of the cellular material without distutbance.

filtrates from plasma of very young calves. The difficulty was overcome somewhat by prolonged standing and refiltration.

4. *Autopsy.* Gross post mortem examinations were made within several hours after death of the experimental animal. Histological studies were made of selected organs, the liver, the kidneys, the spleen and the heart, from representative animals.

EXPERIMENTAL RESULTS

General health. All the calves apparently were healthy and vigorous during the first stages, from 25 to 60 days. Subsequently many symptoms varying in kind and degree were manifested. In general, however, the sequence of changes are in accord with the reports of other investigators (15).

The gross symptomatology common to all the calves was first a depraved appetite as manifested in the insatiable desire to gnaw boards, chew shavings and lick hair. This abnormality was generally followed by the development of a long rough coat of hair, dry harsh skin, paleness of unpigmented areas, emaciation, sluggishness, occasional nervousness and anorexia. Colds and digestive disturbances were frequent and varied in duration and intensity with individual calves.

Some symptoms were restricted to a few individuals. Three of the calves, E–230, E–231 and E–232, experienced difficulty in swallowing. This symptom was very pronounced in the first two calves at 3 months of age but not in the latter until 9 months of age. There seemed to be a lack of coordination and synchronization in the deglutition process, frequently resulting in choking. This difficulty was alleviated somewhat by elevating the pail while the calf was attempting to drink. This symptom persisted until death in the case of E–231, which remained on the exclusive milk diet, and until the diet was changed in the cases of E–230 and E–232.

Other important gross symptoms encountered in various individuals were paresis, incoordinated locomotion as a consequence of lack of control of rear quarters, rachitic-like condition, edema of the joints, profuse lacrymation, excessive salivation, dyspnea, nasal discharges, nervousness, exophthalmus, tonic-clonic convulsions, retarded growth, chronic diarrhea and cachexia. The resistance of the calves to the syndrome varied considerably as suggested

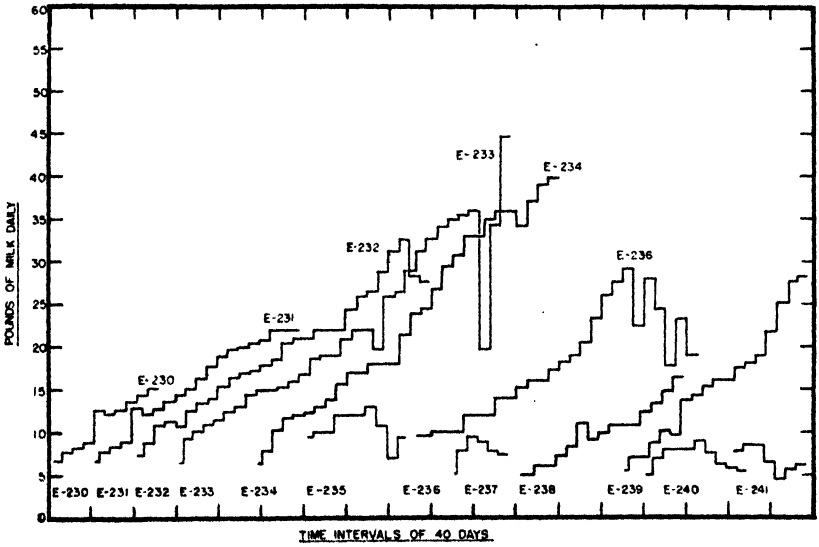

FIG. 2. Average daily milk intake of experimental calves by 10 day periods.

by the differences in the time they remained on experiment, shown in Table 1. The etiology in every case appeared to be of a dystrophic nature.

Milk consumption and growth of calves. The milk consumption, as presented graphically in Figure 2, was decidedly different for various calves. For the animals maintaining a good appetite the absolute daily intake was increased with advancing age, but the intake per unit of body weight actually decreased. Since the appetites of many of the calves were erratic, especially during the last stages of the experiment, the intake from day to day was variable.

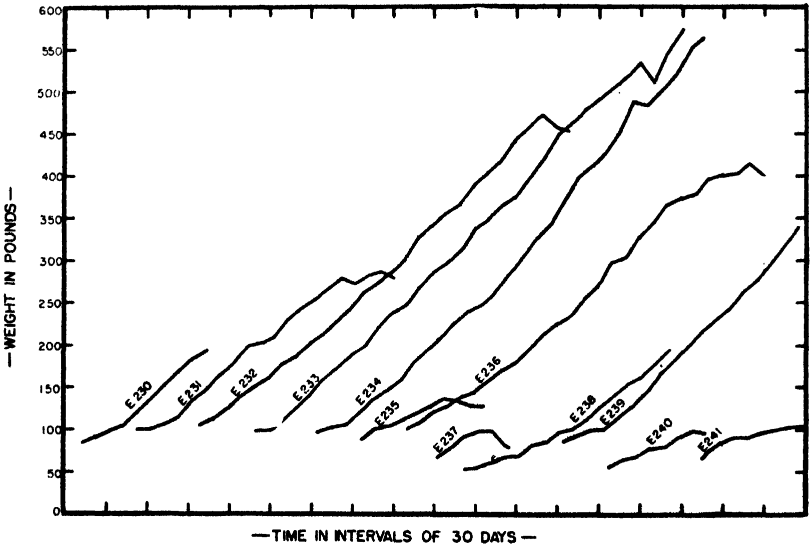

FIG. 3. Growth rate of experimental calves.

Growth, as measured by changes in body weight, is indicated in Figure 3. As with milk consumption, there is also much variation in rate of development. The extremes are probably due to two major factors, first, heterogenity of the experimental animals from a genetic standpoint and second, variation in nutrient intake.

Changes in composition of blood. Blood analyses were initiated too late to include E–230 and the early stages of E–231, E–232, E–233 and E–234. The composition of the blood of E–231 is presented in Table 2, and that of the others in Figures 4 to 8 inclusive.

Hematocrit and hemoglobin values. Anemia and low hematocrit values were pronounced in the advanced experimental stages. A supplementary examination of the blood of E–231 shortly before death revealed the following hematological picture: 2,900,000 erythrocytes and 10,440 leucocytes per ml., and a differential count of 84.5 per cent lymphocytes, 0.5 per cent mono-

TABLE 2

Composition of blood of E-231

Age	Blood			Plasma			
	Red blood cells	Hemoglobin	Sugar	Ca	Inorg. P	Mg	Fat
Days	*vol. %*	*gms. per 100 mls.*	*mgs. per 100 mls.*	*mgs. per 100 mls.*			
149	...	...	115.2	10.72	7.19	...	...
167	...	4.92	127.0	9.94	4.69	...	...
179	...	4.62	112.5	11.15	4.18	...	129.3
186	...	4.05	115.5	10.21	3.80	0.86	108.2
193	...	3.23	120.0	9.99	3.26	0.93	109.3
194	14.17	3.01	167.0	12.01	3.52	0.72	121.9
196*	15.26	2.40	..	12.39	3.40	0.97	47.3

* Died.

cytes and 15 per cent polymorphonuclear cells. Only two calves, E-235 and E-236 (Figs. 5 and 6), of seven calves studied within the first ten days after birth had hemoglobin values that could be considered within the normal range. The remaining five, E-237 to E-241, inclusive, had initial values between 4.43 gms. and 5.72 gms. The hemoglobin of calves having the initial high values gradually decreased to the level of the calves starting low; whereas, with only one exception, the hemoglobin of the calves having the initial low levels exhibited no significant variations. The exception, E-237, starting at the low level of 4.43 gms. attained a lower value of 2.09 gms., the lowest recorded in this investigation.

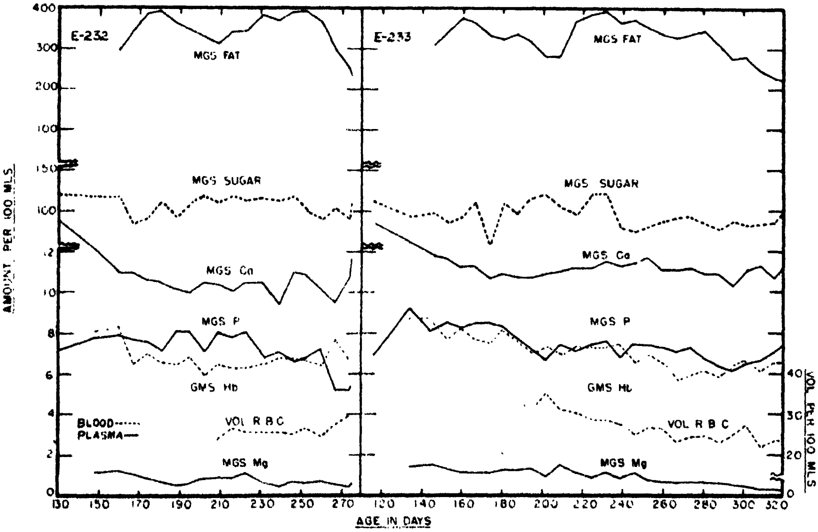

Fig. 4. Changes in composition of blood and plasma of E-232 and E-233.

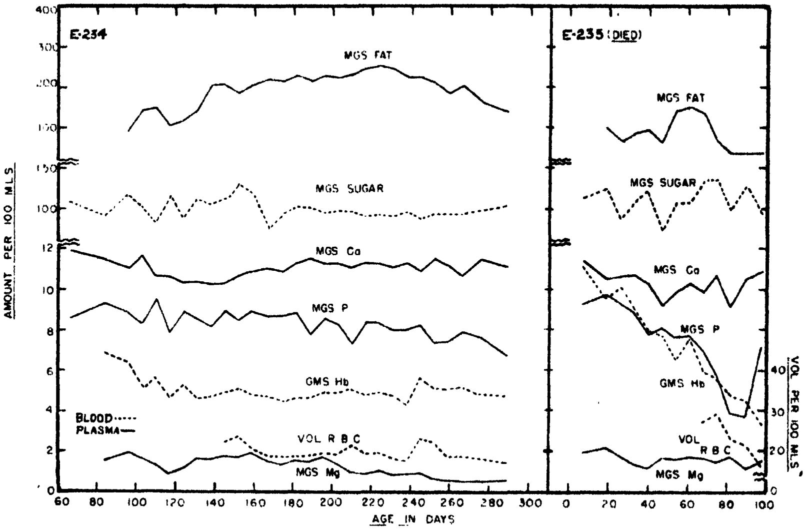

FIG. 5. Changes in composition of blood and plasma of E-234 and E-235.

Blood sugar. The data show that the levels for blood sugar of all calves were high, and that the values varied greatly with different individuals and in the same calf from time to time.

Fat of plasma. There was also a pronounced difference in the fat content of plasma in various calves. Generally the fat gradually increased to 200

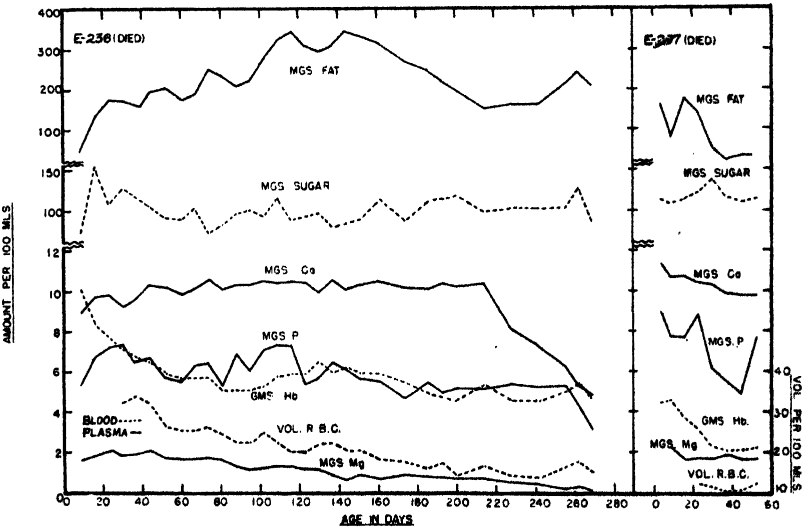

FIG. 6. Changes in composition of blood and plasma of E-236 and E-237.

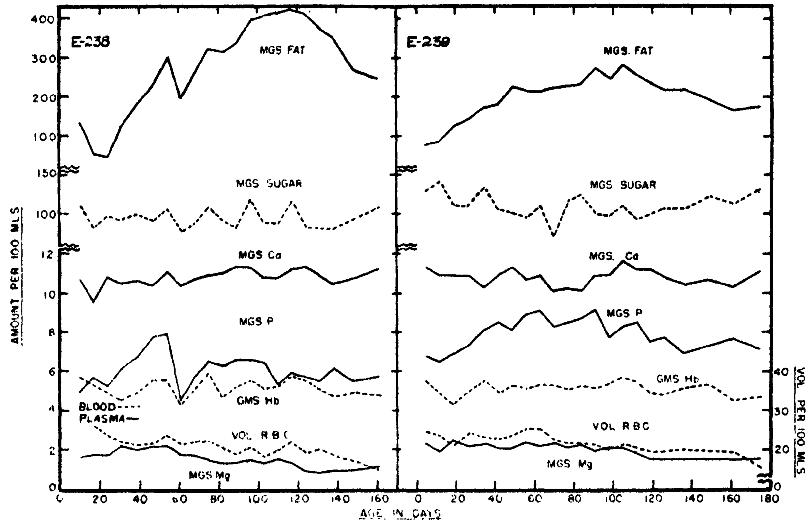

Fig. 7. Changes in composition of blood and plasma of E–238 and E–239.

mg. or more per 100 mls. if the calf remained thrifty. Otherwise the trend was up for a short time then downward and variable. During the early stages of life, the fat in accordance with the observations of Allen (2), was almost nil and practically devoid of pigments. The subsequent degree of pigmentation seemed to depend not only upon the quantity and quality of milk but also upon the individual calf.

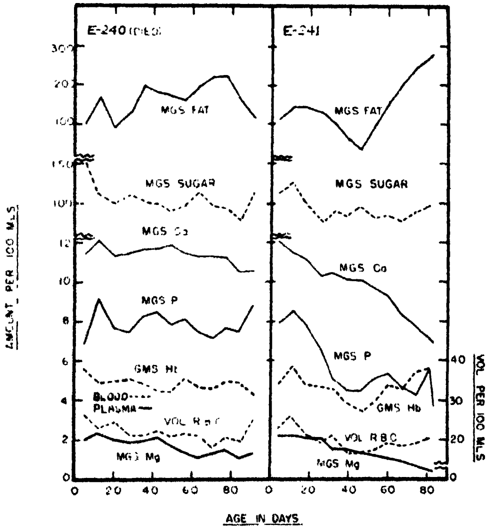

Fig. 8. Changes in composition of blood and plasma of E–240 and E–241.

Calcium of plasma. With the exception of two calves there were no evidences of abnormal calcium values. E–236 and E–241 attained the low values of 4.81 mgs. and 6.86 mgs. per 100 mls., respectively, at which levels both were convulsive.

Inorganic phosphorus of plasma. The inorganic phosphorus likewise varied considerably. As revealed in Figures 5, 6, 7 and 8, the extremes were 4.98 mgs. and 9.24 mgs. per 100 mls. for calves within the first ten days after birth. Subsequent trends were generally upward for a short period followed by a gradual decrease which rarely went below 6 mgs. However, the initial increase in two calves, E–235 and E–237, was followed by a rapid reduction until shortly before death, at which time a marked increase was observed. A gradual downward trend was observed in E–236 and E–241, terminating in tetanic seizures. The phosphorus levels of E–231, shortly before death, and those of E–232, in a semi-paralytic state, were also subnormal.

Magnesium of plasma. The important and perhaps significant fact is that within the first three or four months after birth there was a general decrease in the amount of plasma magnesium. Evidently in the cases of E–231, E–232, E–233 and E–234 a marked reduction of the element had taken place before blood analyses were applied. As may be noted, the rate and degree of reduction again emphasizes the varied responses of individual calves apparently subjected to similar treatment.

Post mortem examinations. Five calves ranging in ages from 55 to 270 days were autopsied. Examination of the digestive system revealed underdeveloped rumens in three cases and normal development in two others. Shavings, bits of curd, particles of loose hair, and hair balls were common ruminal contents. In every case there were lumps of undigested curd in the abomasum. In some cases the pyloric region was ulcerated. Generally the mucosa of the abomasum and the duodenum were slightly catarrhal.

Inspection of other organs revealed an atonic condition of the heart and petechial hemorrhages on the exterior surface of the auricular region in several cases. The spleens were generally atrophic, but the one from E–231 was decidedly hypertrophic. The adrenals in the cases of E–235 and E–236, were somewhat enlarged. Nephritis of calves E–235, E–237 and E–240, which died in an early stage, were characterized by numerous white spots on the kidney surface. With the exception of E–240 the livers were abnormal—anemic in appearance, hypertrophied and showed evidence of fatty infiltration.

The muscle tissue was generally pale and flaccid. The bones of the older animals, E–231 and E–236, both rachitic, showed evidence of osteoporosis on the articulating surfaces. Such symptoms were not evident on the bones of younger calves.

DISCUSSION OF RESULTS

The variability and complexity of the symptomatology render it difficult to assess the true significance of some of the results. The high and variable blood sugar values reported herein are in accord with other results (16, 32) obtained from normal young calves. In agreement with the findings of Allen (2) working with normal animals, individuality and amount of fat consumed play important roles in the fat content of the plasma, but there are apparently additional factors involved. Digestive disturbances, for example, frequently cause marked reductions in plasma fat. Whether or not the metabolism of carbohydrates and fats as suggested by Sjollema (27) may be considered as a major factor in the etiology of the syndrome resulting from exclusive milk feeding remains a moot question. However, the results do not indicate that these two organic compounds play an important role.

The deficiency of iron and copper as manifested by anemia was very pronounced. Contrary to general findings, there were several cases of anemia in very young calves. These results are interesting in view of the belief that new-born animals are supplied with sufficient mineral reserves to tide them over the normal exclusive milk consuming period. However, in harmony with the reports of other investigators (15) normal hemoglobin levels are gradually reduced when the calves are restricted to milk rations. The complication of anemia with other symptoms render it difficult to establish a hemoglobin level below which death is likely to occur, but the data suggest that calves cannot tolerate values much below 3.00 gms. per 100 mls. of blood.

The interrelationship of calcium, phosphorus and magnesium and their relation to other constituents under the various conditions encountered render interpretation of results hazardous. The problems associated with the adequacy of these constituents in a milk diet and the degree of utilization by calves are still controversial.

This seems to be especially true of magnesium. The gradual reduction in the magnesium levels is in accordance with the reports from Michigan (11), Ohio (19) and Kansas (6) but contrary to the Missouri findings (15). A consideration of the theoretical needs of calves (11, 27) and the amount of magnesium in milk (20, 29) suggest that hypomagnesemia is the result of faulty utilization, the causes of which are unknown. Tufts and Greenberg (31) showed that diets high in calcium shorten the life span and increase the incidence and severity of the convulsive attacks of magnesium deficient rats. It is hardly conceivable that the Ca : Mg ratio in milk can be a major factor affecting the magnesium utilization by calves.

The hypocalcemia and the hypophosphoremia evidently were due to inefficient utilization of these elements. This might be attributed partly to an inadequacy of vitamin D (21) in the diet, especially during the winter season. Since diarrhea was frequently accompanied by a sudden drop in inorganic phosphorus it seems that digestive disturbances interfered with either absorption or retention of this element. In several cases there seemed to be a close relation between the trends of the phosphorus and the hemoglobin and the volume of erythrocytes. Though possible, the evidence is inadequate to suggest that an anemic condition may interfere with phosphorus metabolism.

It is difficult to ascribe any one of the multitude of gross symptoms specifically to the changes of any one constituent. Anemia and hypomagnesemia were common to all the calves but in different degrees. Though it has been demonstrated that tetany of magnesium deprivation is not due to an alteration in the calcium ion concentration of the blood (17), the tonic-clonic convulsions observed in E–236 and E–241 involved hypocalcemia and hypophosphoremia in addition to anemia and hypomagnesemia. Also the two cases showing rachitic symptoms were different as indicated by the calcium and the inorganic phosphorus of the plasma. For E–231 it was normal calcium—low phosphorus; whereas for E–236 it was low calcium—low phosphorus. The sudden increase of inorganic phosphorus following prolonged cachexia and shortly preceding the deaths of E–235, E–237 and E–240 may be attributed to initiation of protoplasmic disintegration liberating phosphorus into the plasma. These conditions are indicative of the complexity of the symptomology encountered.

It is likewise impossible to point to the specific factors responsible for the pathology of various internal organs. The so-called "spotted kidneys" observed in E–235, E–237 and E–240 may be attributed to either vitamin A deficiency (18) (22) (28) or to magnesium deficiency (8) (14) (23). The early appearance of the nephritic condition, the chronic digestive disturbances, the relatively high magnesium values of the plasma and the general unthrifty appearance are provisional evidence for listing vitamin A deficiency as the probable predisposing factor in the pathology of these calves.

Probably the cases of sudden change in the color of the plasma, from opalescence or very light yellow to a greenish brown, shortly before or during exhibitions of drastic physiological disturbances may be associated with the dysfunction of one or more organs. Brunner (5) reported that urobilemia produced a green fluorescence of the blood. Bollman and Schlotthauer (4) stated that an accumulation of bilirubin in the serum is observed when the liver or the bile ducts are infected. Though the evidence is very superficial, it is possible that liver and/or kidney disturbances were instrumental in the plasma pigmentation.

Another symptom that cannot be overlooked, is the general catarrhal enteritis and gastritis which obviously interfere with assimilation and subsequent metabolism. It remains to be determined whether the disturbances are due to the physical state of the milk in the digestive tract as suggested by Sheehy (26), to an inherent deficiency of the diet, or to an excess of some constituent in the milk.

SUMMARY AND CONCLUSIONS

When dairy calves, managed as outlined, are restricted for a prolonged period to a whole milk ration, the following abnormalities may be observed at various times:

1. General unthrifty appearance, anorexia, cachexia, dyspnea, occasional nervousness and tetany, rachitic condition, dysphagia, and digestive disturbances.
2. Marked variations in the growth rate.
3. Changes in the constituents of the blood involving high but variable values for blood sugar, extremes in fat of plasma but generally high values, decrease in hemoglobin, gradual downward trend of magnesium of plasma, occasional cases of hypophosphoremia, and rarely cases of hypocalcemia.
4. Abnormal condition of several internal organs—hypertrophied livers in which fatty degeneration is taking place, gastritis, enteritis, and nephritis.

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VALUE OF VARIOUS VITAMINS AND INORGANIC SALTS FOR OVERCOMING SYMPTOMS MANIFESTED IN CALVES RESTRICTED TO A WHOLE MILK RATION[1]

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Since the discovery of the value of minerals and vitamins for growth and health, and even for life itself, many investigations have been conducted to ascertain the essentiality of these constituents in rations of cattle. Even with the vast amount of information acquired, the knowledge of the quantitative and qualitative needs of calves is still fragmentary. The dearth of specific information relative to the curative properties of various supplements when administered to calves manifesting dystrophic symptoms resulting from milk feeding provoked further investigation.

Previous results (23) revealed that in the syndrome developed from restricting calves to a whole milk ration anemia and hypomagnesemia were common. Less frequent were evidences of avitaminosis A and D. These symptoms in accordance with the results of other investigators as reviewed by Herman (9) suggested the advisability of adding vitamins, A and D, and the minerals, iron, copper and magnesium to the milk ration. Thus the primary object was to determine the responses of these malnourished calves to the addition of the foregoing constituents, individually or in combination.

EXPERIMENTAL PROCEDURE

The subjects, Table 1, were malnourished calves transferred from a previous experiment (23). The care and the management of these calves were virtually the same as already described (23). During spring, summer and early autumn two of the animals, E–233 and E–234, were released for exercise, water and sunlight into a small yard covered with gravelly earth and cinders but free of vegetative material.

The mineral elements were provided in the following salts: copper in cupric sulphate; iron in ferric chloride; magnesium in magnesium carbonate, magnesium chloride or magnesium sulphate; iodine in potassium iodide. A solution of cupric sulphate and ferric chloride was prepared so that the ratio of iron to copper was 10: 1. The concentration was such that 1 ml. of solution contained 100 mgs. of iron and 10 mgs. of copper. Other solutions

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TABLE 1

Description of calves; supplements fed; and duration of experimental treatment

No. of calf	Started on experiment		Supplements in order of addition	Removed from experiment	
	Age	Pronounced physiological state		Age	Cause
	days			*days*	
E-230	104	Lethargic; dysphagia	Magnesium sulphate	188	Death
E-232	274	Paresis; dysphagia	Magnesium chloride	331	Death
E-233	325	Hyperirritable; stiff	(1) C.L.O. + (2) $MgCO_3$ + (3) $FeCl_3$ & $CuSO_4$ soln.	720	Death
E-234	290	Anemic; hypomagnesemic	(1) $FeCl_3$ & $CuSO_4$ soln. + (2) $MgCO_3$ + (3) C.L.O.	555	Death
E-238	162	Anemic; exophthalmus	(1) $FeCl_3$ & $CuSO_4$ soln. + (2) $MgCO_3$ + C.L.O. + glucose	247	Sold
E-239	182	Anemic	(1) $FeCl_3$ & $CuSO_4$ soln. + (2) $MgCO_3$ + C.L.O.	235	Sold
E-241	83	Convulsive; emaciated; anemic	(1) C.L.O. + (2) $MgCO_3$ + (3) $FeCl_3$ & $CuSO_4$ soln.	234	Transferred

were 10 per cent magnesium sulphate and 50 per cent magnesium chloride, including water of crystallization in both cases. The low consumption of iodized salt prompted the inclusion of iodine which was prepared as a 1.3 per cent solution of potassium iodide. Since the solubility of magnesium carbonate in water is very low, the daily allotment of this salt for each calf was weighed in individual paper envelopes.

At the time this work was initiated, there were reports indicating that U.S.P. cod liver oil fed at moderate to high levels may be injurious to calves (6, 15, 21). In order to eliminate this possibly deleterious factor the concentrated oil, "Nopco XX," was fed.

Little information relative to the amounts of the foregoing supplements needed by cattle is available. The quantity of iron and copper provided was essentially the same as recommended by Knoop, Krauss and Washburn (13). When this investigation was started, the only suggestion of the quantitative needs of magnesium was the amounts supplied in normal rations (1). The standard arbitrarily adopted was to provide sufficient amounts of magnesium, usually magnesium carbonate, to maintain the level of the plasma magnesium between 2.0 and 2.5 mgs. per 100 mls. As a precautionary measure against possible iodine deficiency, 5 mls. of the 1.3 per cent potassium iodide solution were fed daily.

The manner of administration was usually orally, but in exceptional cases injection was employed in order to accelerate the reaction. The oral administration was made by adding the supplements directly to the milk except when the animals had erratic appetites. In these cases the material was suspended or dissolved in water and given by drenching. The inorganic supplements were fed at morning feeding and the organic at evening.

The interval for drawing blood samples was extended from one week to two weeks.

EXPERIMENTAL RESULTS

The major consideration is the responses of the various calves to the supplements as manifested in general health and in the changes of blood composition, shown graphically in Figures 1 to 3, inclusive. As indicated in Table 1, there was little uniformity of animals transferred to this experiment. Likewise the subsequent reactions varied.

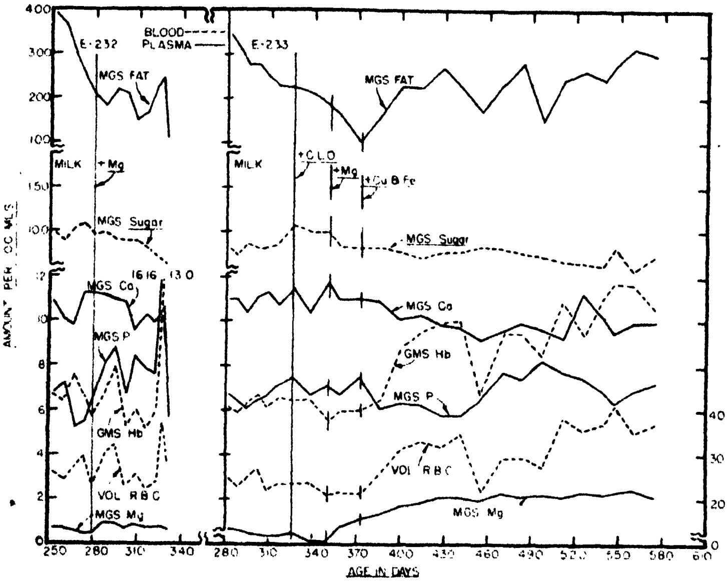

FIG. 1. Changes in composition of blood and plasma of E–232 and E–233.

Two calves, E–230 and E–232, received only magnesium supplements. The first, E–230, received 50 mls. of a 10 per cent solution of magnesium sulphate daily. The only observable changes were amelioration of the swallowing difficulty and increased vigor. Death ensued later following the sudden onset of a disturbance involving anorexia, constipation, abdominal distention and dyspnea. The increased concentration of the inorganic constituents of the blood, Table 2, and the development of a yellowish plasma pigment (23) shortly preceding death indicated marked physiological changes. Post mortem examination revealed a vast amount of fluid in the digestive tract but practically no liquid in the bladder. It appeared as

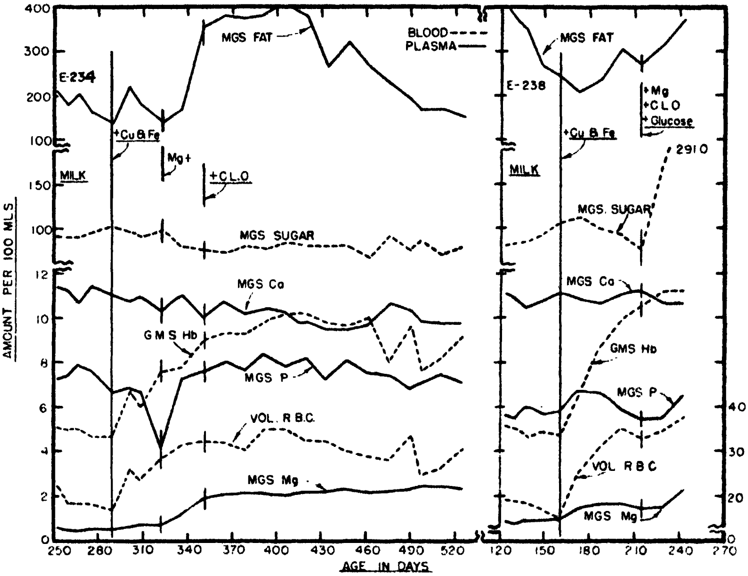

Fig. 2. Changes in composition of blood and plasma of E-234 and E-238.

though there had been a migration of fluids from the body tissues into the alimentary canal.

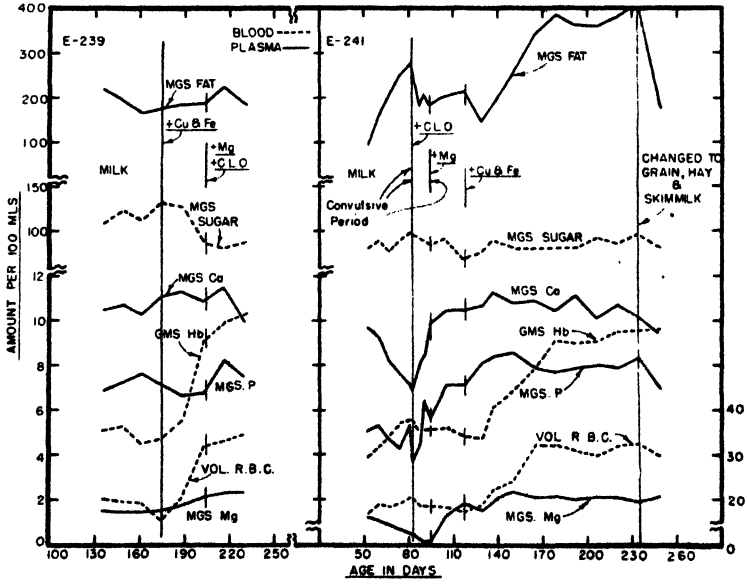

Fig. 3. Changes in composition of blood and plasma of E-239 and E-241.

TABLE 2

Composition of blood of E–230

Age	Blood		Plasma			
	Amount per 100 mls.		Amount per 100 mls.			
	Hemoglobin	Sugar	Ca	Inorg. P	Mg	Fat
days	*gms.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*
154	......	113.9	10.56	7.81	......	....
172	4.82	116.7	13.40	7.72	1.21	....
184	5.41	111.5	11.74	8.08	0.97	313.5
188[1]	7.35	87.0	13.41	11.41	2.52	290.2
188[2]	......	107.0	16.62	19.73	3.98	300.8

[1] Sample of blood drawn 2.5 hours before death.

[2] Sample of blood drawn 0.5 hour after death.

In the case of E–232 the syndrome included dysphagia, stiffness, enlarged joints and paresis accompanied by anemia and hypomagnesemia, Figure 1. In an attempt to relieve some of the symptoms, paresis particularly, several intravenous injections of a 10 per cent solution of magnesium chloride were made. This treatment aided in overcoming the semi-paralytic state. Subsequently magnesium chloride was administered orally. Dysphagia was relieved, but other disorders grew progressively worse. Digestive disturbances were frequent, especially following increases of the dosage of the supplement. The magnesium of the plasma was altered very little by the increased magnesium intake. In the final stages of life the animal acquired a dehydrated appearance, became very stiff, manifested alterations in blood including increased viscosity and pronounced changes in composition, Figure 1. Diarrhea and bloat, from gaseous putrefaction of a large amount of curd compacted in the rumen, apparently were the direct causes of death.

The supplements given to E–233, indicated in Table 1 and Figure 1, aided in relieving nervousness, stiffness and lassitude but did not overcome an erratic and depraved appetite. The elevation of the plasma magnesium level, following the addition of magnesium carbonate to the diet, and the increases of hemoglobin and red blood cells, following iron and copper supplementation were very marked. The sudden drop of the hemoglobin and the red cells on the 456th day was the result of a hemorrhage resulting from the removal of a large wart. Normal reproductive functions were evidenced by regular oestrus, by conception to second breeding, and by the presence of a well developed foetus (202 days of age) at the time of autopsy. The general health of the animal apparently was good until death at 720 days of age. The fact that a normally fed heifer in the same annex of the barn as E–233 died suddenly two days previously suggested that the cause of the death of E–233 was foreign to the experimental conditions. The post mortem examination revealed no abnormalities sufficient to cause death.

The responses of E–234 were similar to those of E–233. However, E–234 failed to conceive, but, as revealed in autopsy, her reproductive organs were normal. Though both animals possessed a depraved appetite, it was more marked in E–234. The excessive consumption of sand, gravel and cinders from the exercise lot produced unassessable damage that counteracted the recognized beneficial effects of sunshine and exercise. So much undigestible foreign material passed into the alimentary canal that rumenitis, acute gastritis and enteritis developed causing death at 18 months of age. Some nutritional deficiency might have been involved, but the mechanical irritation (1900 gms. of sand in the abomasum alone) was sufficient to initiate and aggravate the malady.

The remaining calves, two males and a free-martin, were removed from experiment as soon as they returned to normal. The two males, E–238 and E–239, responded quickly and profoundly to the supplementation. It is interesting to note the increase of the plasma magnesium following the addition of iron and copper. This response was also observed to a less extent in E–234. The increase may be attributed in part to the slight increase of magnesium intake resulting from a greater milk consumption. It is also conceivable that the supplements affected a more efficient utilization.

While E–241 was in the throes of a tonic-clonic convulsion, the blood composition was as shown in Figure 3. Calcium chloride was injected intravenously. This gave temporary relief probably as a consequence of a direct increase of calcium and an indirect increase of magnesium (19). Though calcium and inorganic phosphorus increased to normal following the addition of cod liver oil to the ration, convulsions persisted intermittently until magnesium was administered—first magnesium chloride intravenously followed by magnesium carbonate per os. This was the only direct positive evidence indicating that hypomagnesemia alone may be responsible for tetany (8). As in other cases, the response to copper and iron also was marked. Growth ceased during the period of the syndrome but resumed when the blood returned to normal composition.

Apparently neither the fat of the plasma nor the sugar of the blood was affected directly by the cod liver oil and the inorganic supplements. Though the fat frequently increased, this was the result of increased intake of milk and improvement of general health of the animal.

DISCUSSION

The addition of the minerals, copper, iron and magnesium, to the milk ration of calves manifesting symptoms of nutritional deficiency brought about profound changes in the health of the animals, in the hemoglobin of the blood and in the magnesium of the plasma. Additional supplementation with vitamins A and D, cod liver oil, no doubt contributed much to the improvement of health but induced no recognizable changes in the blood other than the increase of calcium and phosphorus when these constituents were low. All the aforementioned supplements seemed to be essential for recovery and maintenance of health, particularly during the winter season.

The changes of the fat of the plasma and the sugar of the blood before (23) and following supplementation of the milk ration suggest that the metabolism of these two constituents are not directly involved in the syndrome. However, the level of the fat seems to vary with the intake of milk and the general health of the calves.

Of the many constituents considered in the etiology of the milk syndrome of calves, magnesium remains in the controversial limelight. The indispensability of this constituent in the diets of the animal organism is definitely established (19), but the amount needed by various species is unknown. This introduces the question of whether or not milk, when consumed to the extent of the appetite of a normal calf, supplies adequate magnesium for maintenance of normal functions. With one exception (9) the present evidence (3, 8, 11, 12, 23) indicates that calves consuming a milk ration that is not supplemented by magnesium become hypomagnesemic.

The etiological factor or factors involved in lowering the plasma magnesium constitute the crux of the problem. Neither Duncan and associates (8) nor Sjollema (20) attributed the lowered magnesium to an inherent deficiency of the milk but to a failure in the utilization of the available magnesium. Likewise Cunningham (5) has concluded, on the basis of the magnesium content of various feeds, that dietary deficiency is not the cause of the hypomagnesemia associated with grass staggers. Though the weight of evidence is against the postulation of an actual inadequacy of magnesium in milk, this factor cannot be dismissed until more is known of the absolute mineral needs of the calves. The intake of magnesium for calves consuming normal grain and hay rations (1) is about five times that of a similar calf receiving a whole milk ration. This obviously does not signify that the calf needs all the magnesium provided in the ration, but suggests the possibility of calves needing more magnesium than is now suspected. The high magnesium content (4, 12) of the bones of normal calves, and the stimulation of growth by milk also suggest the possibilities of increased needs of magnesium.

Perhaps the amount of magnesium normally eliminated is a factor that should be considered further in the adequacy of this constituent in the ration. Hirschfelder and Haury (10) stated, "normal animals and humans excrete about 42 per cent of ingested magnesium in the urine in 24 hours. The percentage of magnesium is fairly constant and is independent both of the dose and of the concentration of magnesium in the plasma." Boyd and Courtnay (2) observed that in the human subject all types of nephritis were accompanied by excessive loss of magnesium from the body. The nephritic condition of calves (17, 23) associated with hypomagnesemia may be either the cause or the result of the decreased magnesium. Until further information is forthcoming, especially from metabolism trials, both the quantity of magnesium in the milk and the degree of utilization must be considered as etiological factors.

Regardless, the striking fact is that the addition of magnesium carbonate to whole milk rations of hypomagnesemic calves results in the restoration to the normal magnesium level. Huffman and Duncan (11) found that it was necessary to supplement milk with 15 to 20 mgs. of magnesium as magnesium oxide, per pound of body weight to maintain plasma magnesium values at normal levels. Only 8 to 10 mgs. were necessary when the source was natural feeds. In this investigation, after the calves recovered, a daily supplemental intake of five grams of powdered magnesium carbonate per 100 pounds of body weight (12.4 mgs. of Mg per lb. of weight) was adequate to maintain the plasma magnesium at a level not less than 2.00 mgs. per 100 mls. of plasma. The total daily intake per pound of body weight generally ranged from 13.0 to 18.0 mgs.

The only abnormality that was not overcome by incorporating the foregoing supplements in the milk was the depraved and erratic appetite. The adequacy of the supplemented milk ration for maintenance of other normal functions throughout a complete life cycle of the bovine remains unsolved. Unfortunately the results from the two subjects, E–233 and E–234, selected to test the permanency of the recovery from the deficiency syndrome were somewhat vitiated by the peculiar circumstances under which their deaths occurred.

Other minerals not included in the ration may be essential for sustenance of normal life functions. Since manganese, of which milk is known to be deficient, has not been demonstrated to be indispensible in the rations of cattle, this element was omitted. It is possible that this deficiency might have affected some important hitherto unrecognized metabolic functions. However, the essential rôle of manganese according to Von Oettingen (22) appears to be associated with reproductive cycles. According to Neal and Ahmann (18) cobalt is also an essential element in the rations of cattle. Calves receiving a basal ration of which dry skimmilk was a constituent manifested deficiency symptoms that were amenable to cobalt supplementation. This in conjunction with the fact that the presence of cobalt in milk has not been demonstrated (7) indicates that this mineral probably should be considered among the inadequate substances.

Other constituents including potent grass juice (14) and vitamin B_4 (9) have been proposed as supplements for raising calves on a liquid diet. The latter deficiency, vitamin B_4, was considered as a possible predisposing cause of chronic catarrhal gastritis and enteritis and ulceration of the pyloric mucosa. It is conceivable that some phase of the vitamin B-complex may be involved. A study of the problem evokes several questions. Does the calf need the vitamin B-complex for normal physiological functions? If so, as applied to the problem of sustained milk feeding, does raw milk supply a sufficient quantity? Since Mangold and Usuelli (16) have demonstrated that milk rations cause the death of rumen infusoria, is it possible for the calves to synthesize the complex when they are restricted to whole milk rations? Until these questions are answered the vitamin B-complex cannot be disregarded as a factor in the problem under consideration.

SUMMARY AND CONCLUSIONS

1. Nearly all the recognizable symptoms of the syndrome developed in calves restricted to whole milk rations may be temporarily remedied by supplementing the diet with ferric chloride, cupric sulphate, magnesium carbonate and cod liver oil.
2. The adequacy of the supplemented ration for sustained normal functions throughout a life cycle including development, reproduction and lactation has not been demonstrated beyond the stage of advanced gestation.
3. The most pronounced abnormality that persisted after supplementation was a depraved and erratic appetite, which might have been the consequence of either a dietary deficiency or the physical nature of the ration.
4. The remedial results indicate that all the foregoing supplements are essential, but reveals little relative to fundamental etiology of the syndrome.
5. In view of the present evidence, it is probable that additional factors should be considered in the etiology of the pathological syndrome resulting from the inadequacy of milk rations for cattle.

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THE INFLUENCE OF THREE METHODS OF CLEANING THE CREAM SEPARATOR AND CREAM STORAGE TEMPERATURE ON THE KEEPING QUALITY OF CREAM[1]

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INTRODUCTION

There is ample evidence in the literature to show that the use of an unwashed separator will result in highly contaminated cream from a bacteriological viewpoint. Great emphasis has been placed upon the importance of properly cleaning and sterilizing the separator after each time used. Under practical farm conditions, however, such an ideal procedure is frequently difficult to attain.

On many cream producing farms the general practice is to wash the separator but once daily. Improving the sanitary condition of the separator on the average farm is perhaps not so much a problem of education as it is in finding a procedure which will reduce the time and labor involved in cleaning the separator. Such a method has been proposed by Rogers and Evans.[2] Their method consists in immersing the separator parts continuously in a solution of trisodium phosphate and sodium chromate when not in use. Before immersing the parts in the solution they are rinsed with clean water and again when removed just prior to assembling the separator. No other cleaning treatment is involved.

Rogers and Evans reported this procedure reduced bacterial contamination of cream to a practical minimum. No serious corrosion of the metal parts resulted from the treatment. The effect of such a cleaning procedure on the flavor or keeping quality of cream was not determined. It would seem that such should be done before the method is generally recommended.

The principal objective of the investigation herein reported was to compare the effects of three procedures of cleaning and sterilizing the separator parts on the flavor and keeping quality of cream when held at three different storage temperatures.

PROCEDURE

In each of a series of nine trials split batches (10 gallons each) of whole milk were separated through three different separators, each of which had been cleaned by a different procedure. The three methods of cleaning cream separators compared in this study were: (1) Conventional, (2) Rogers, and (3) Unwashed.

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The conventional method consisted in thoroughly washing and steam sterilizing the separator parts before use. In the case of the Rogers method, the separator parts were immersed continuously when not in use in a solution composed of 5.0 pounds of trisodium phosphate, 0.25 pounds of sodium chromate, and 100 pounds of water. Except for rinsing the parts with a stream of water from a hose before placing them in the solution and again before assembling the separator no further cleaning treatment was involved. The unwashed method consisted in separating five gallons of whole milk through a clean separator on the day prior to the beginning of each trial and then leaving the separator unwashed over night.

The parts of each separator were cleaned by the same procedure for three consecutive trials and then were alternated so that during the course of the nine trials each separator was subjected to all three methods of cleaning. In this way it was possible to observe any effects of the cleaning treatment on the different metals used in the construction of the separator. In one of the separators all parts were constructed of stainless steel, another was equipped with stainless steel discs, whereas the third machine was constructed of tinned iron or steel throughout.

Each series of three trials extended over a two weeks period, during which the parts of the separator being cleaned by the Rogers method were immersed continuously in the solution when not in use, the same cleaning solution being employed for the entire period. To observe any accumulation of organic matter in the solution over a period of two weeks and to note whether the continued additions of organic material to the solution would have any influence on the effectiveness of the treatment, ten gallons of whole milk were separated daily and the parts placed in the solution after rinsing with water but not washed. Cream samples for comparative purposes, however, were taken only at the beginning and after one and two weeks respectively.

Each separator was adjusted to deliver cream testing 30–35 per cent fat. Each of the three lots of cream obtained was subdivided into three portions, placed into clean sterilized two quart glass fruit jars and stored in incubators adjusted to operate at 60, 70, and 80° F. respectively.

The various lots of cream were held at the temperatures indicated until they became second grade. Samples were removed daily, numbered so as to conceal their identity, and graded independently by two judges. The definitions given in the Kansas State Dairy Law for cream grades were used as a basis for grading. First grade cream is defined as cream that is clean, smooth, free from undesirable odors, clean to the taste and sweet or only slightly sour. Second grade cream is described as cream that is too sour to grade as first, that contains undesirable flavors and odors in a moderate degree that is foamy, yeasty or slightly stale, or that is too old to pass as first grade.

After the samples had been graded the acidity calculated as lactic acid was determined by titrating a nine gram portion with 0.1 normal NaOH using phenolphthalein as an indicator.

Immediately following the determination of the acidity 10 ml. of commercial formaldehyde, previously neutralized to the end point of phenolphthalein, was added to the cream and the mixture again titrated to the end point of phenolphthalein with 0.05 normal NaOH. This titration, known as the formol titration, is expressed arbitrarily as the ml. of 0.05 normal NaOH required to neutralize the carboxyl groups freed as a result of union of formaldehyde with the amino groups of proteins decomposed by biochemical processes.

RESULTS

Grades. It required from 7–11 days for the cream stored at 60° F. to become second grade when a clean separator was used, 5–12 days when cleaned by the Rogers method and from 2–9 days when an unwashed separator was used (Table 1). From the standpoint of keeping quality of the cream, cleaning the separator by the Rogers method proved to be practically as effective as washing and steam sterilizing the parts just before use and was decidedly superior to the use of an unwashed separator. The use of an unwashed separator as compared with a clean machine reduced the average time required for the cream to change grade nearly three days when stored at 60° F.

TABLE 1

Days required for cream to change from first to second grade as influenced by the cleanliness of the separator and storage temperature (nine trials)

Method of cleaning separator	Temperature of storage					
	60° F.		70° F.		80° F.	
	Range	Average	Range	Average	Range	Average
Conventional	7–11	9.3	4–9	5.8	2–4	3.3
Rogers	5–12	8.5	2–8	5.5	3–4	3.4
Unwashed	2–9	6.5	1–7	3.6	1–4	2.5

The influence of the cleanliness of the separator on the keeping quality of cream was most apparent at the lower storage temperatures. When stored at 80° F. all of the cream became second grade in a relatively short time, averaging 2.5–3.4 days, irrespective of the procedure employed in cleaning the separator.

Temperature of storage was found to exert a greater influence on the keeping quality of cream than did the method of cleaning of the separator. Cream obtained with an unwashed separator remained first grade an average of 6.5 days when stored at 60° F., whereas cream from a clean separator remained first grade for only 5.8 days when stored at 70° F., and for only 3.3 days when stored at 80° F.

TABLE 2

Frequency of occurrence of flavor criticisms of cream samples at the time they were graded as second grade as influenced by the method of cleaning the separator and storage temperature (nine samples in each case)

Flavor criticism	Temperature of storage								
	60° F.			70° F.			80° F.		
	Method of cleaning the separator								
	Conventional	Rogers	Unwashed	Conventional	Rogers	Unwashed	Conventional	Rogers	Unwashed
High acid	3	3	5	9	5	4	6	7	5
Bitter	3	5	8	2	1	3	1	1	0
Yeasty	0	0	0	1	3	2	3	3	3
Unclean	1	1	3	1	0	3	0	3	3
Old stale	3	3	0	1	1	0	1	1	1
Metallic	2	0	1	1	1	0	1	0	1
Musty	1	0	0	0	2	2	0	0	0
Putrid	0	1	0	0	0	0	0	0	0
Cheesy	1	0	0	0	0	0	0	0	0

Flavor criticisms. The specific off-flavors together with the frequency of their occurrence in each of the different lots of cream at the time it was officially graded as second grade are recorded in Table 2. Since more than one flavor criticism was frequently applied to the same sample of cream the number of flavor criticisms exceeds the number of cream samples involved (Table 2).

The types of off-flavors which developed in the cream obtained from the separators cleaned by the Rogers method were no more objectionable than those which developed in the cream produced by the clean separators. Cream samples from the unwashed separator were criticized as "unclean" and "bitter" more frequently than the cream obtained from the separators cleaned by the two other methods. Aside from these two flavor criticisms, however, no definite trend in off-flavor development was observed to be associated with the method of cleaning the separator.

Temperature was found to have a marked influence on the type of off-flavors which developed in the cream during storage. The samples stored at 60° F. developed bitter flavors more frequently than at any other storage temperature. Yeastiness, on the other hand, was observed only in samples stored at 70 and 80° F., and was most frequent at the higher temperature. Samples criticized as "high acid" were usually those stored at the higher temperatures. Musty flavor occurred most frequently in the samples stored at 70° F. The storage temperature, therefore, not only has a marked influence on the time required for off-flavors in cream to develop but it may also govern the specific type of off-flavor.

Acidity. During the first 4 to 5 days of storage at 60° F. the cream obtained from the dirty separators increased in acidity at a faster rate than that obtained from the machines cleaned by the other two methods (Fig. 1). The greatest difference was observed after a two-day storage period. After the fifth day of storage at 60° F. there were no significant differences in the acidities of any of the samples.

As the storage temperature was increased from 60° F. to 70 and 80° F. the rate and extent of acid development in all cream samples was greatly accelerated irrespective of the procedure followed in cleaning the separator. The importance of using both a clean separator and low storage temperature for the production of high quality cream is emphasized by these results.

Formol titrations. The temperature of storage was found to have a greater influence on the rate of proteolytic decomposition, as measured by the formol titration, than did the method of cleaning the separator (Fig. 1). At each of the different storage temperatures the formol titrations were very similar for each lot of cream. As the temperature of storage was increased, however, from 60 to 70 and 80° F. the rate of proteolytic decomposition was accelerated in all samples irrespective of the method employed in cleaning the separators (Fig. 1).

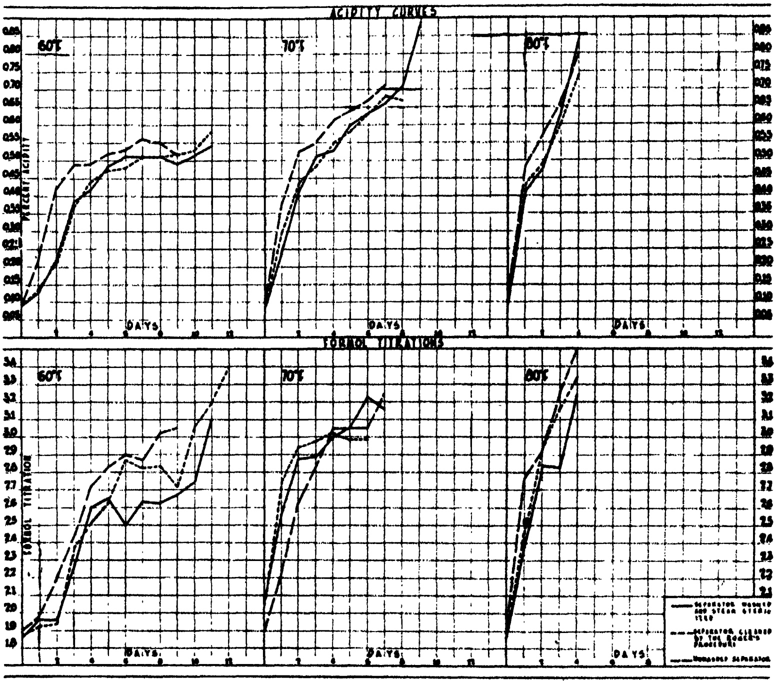

Fig. 1. Effect of three methods of cleaning the separator and temperature of storage on the titratable acidity and formol titration of cream

Effect on metals. Continuous immersion for two weeks in the trisodium phosphate, sodium chromate solution at a temperature of 85–92° F. had no visible effect on the separator parts constructed of stainless steel.

Similar treatment of the separator parts constructed of tinned steel showed some dark discoloration at the end of the two weeks period. The dark discoloration was particularly noticeable at soldered joints, although some of the discs showed discolored areas. The joints around the cream and skim milk spouts and feed cup were most seriously affected.

Strength and appearance of the solution. There was no appreciable depletion in the alkalinity of the trisodium phosphate, sodium chromate solution during a period of two weeks as measured by titrating a 5 ml. sample with 0.10 normal HCl solution using phenolphthalein as indicator. A reduction from 8.0 to 7.5 ml. of a 0.1 normal HCl solution was the greatest change in alkalinity observed in any of the trials. Such a slight change is of no practical significance as far as the cleaning and sterilizing properties of the solution are concerned. Aside from a slightly cloudy appearance at the end of the two weeks period no visible change was observed in the solution.

In no case was any objectionable odor observed nor was there any accumulation of organic material in the solution even though no attempt was made to remove completely the organic material from the separator parts before placing them in the solution.

SUMMARY AND CONCLUSIONS

Comparison has been made of three different procedures ((1) Conventional, (2) Rogers, and (3) Unwashed) of cleaning cream separators on the flavor and keeping quality of the resulting cream when stored at 60, 70, and 80° F. respectively. The data are based upon nine trials. The results are summarized as follows:

1. It required from 7–11 days for the cream stored at 60° F. to become second grade when a clean machine was used, 5–12 days when cleaned by the Rogers method, and from 2–9 days when an unwashed separator was used (Table 1).
2. The temperature of storage was found to exert a greater influence on the keeping quality of the cream than did the method of cleaning the separator. At 80° F. all of the cream samples changed from first to second grade in a relatively short time, averaging 2.5–3.4 days, irrespective of the procedure employed in cleaning the separator.
3. Samples of cream produced with the unwashed separator were criticised as having "unclean" and "bitter" flavors more frequently than those obtained from the other two separators. Cream obtained from the separators cleaned by the Rogers procedure did not develop off-flavors that were any more objectionable than those which developed in the cream produced by the clean separators.
4. Temperature of storage was found to have a significant influence upon the time required for off-flavors to develop in cream and also upon the specific type of off-flavor noted.
5. Temperature of storage was found to have a greater influence upon the rate of acid development and proteolytic decomposition in cream than did method of cleaning the separator.
6. Continuous immersion of stainless steel separator parts in the trisodium phosphate, sodium chromate solution for a period of two weeks at a temperature of 85–92° F. had no visible effect on the metal, but similar treatment of tinned steel or iron separator parts resulted in some dark discoloration particularly at soldered joints.
7. The daily additions of organic matter to the solution used in the Rogers method of cleaning over a two weeks period had no significant influence on its alkalinity nor on the color or condition of the solution.

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A COMPARISON OF THE VITAMIN A POTENCY AND CAROTENE CONTENT OF DIFFERENT TYPES OF SILAGE[1]

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The importance of vitamin A in the ration of the dairy cow has become increasingly evident in recent years and numerous assays have been made of the constituents of the ration. Feeding practices have changed in that prominence is being given to the use of artificially dehydrated hays and new types of silage instead of field cured hays, and consequently many studies have been carried out to determine the effect of these types of processing upon the vitamin A content of the crops. In much of this work a determination of the carotene content has been the method of assay since it has been shown that carotene is the main source of vitamin A in the feeding materials used.

REVIEW OF LITERATURE

It has been found in this laboratory (10) as well as in others (3, 6, 11) that the carotene content of the silage is often markedly higher than that of the fresh green plants from which the silage was prepared. However, no adequate explanation of this anomaly has been given. Peterson and coworkers (6) have advanced the theory that the action of acids which are present in silage, particularly in that prepared by the A.I.V. process, tended in some way to make the carotene present more readily extractable. Kane, Wiseman, Hartman and Cary (2) have reported that the acids present caused the formation, probably from xanthophyll, of a pigment which could not be readily separated from carotene by the usual partition methods, and which rendered inaccurate the spectrophotometric determination of carotene. In a recent investigation, Shinn, Kane, Wiseman and Cary (9) tested carotene extracts from fresh crops, hays, and silages. They found that, particularly in the last two materials, the carotene extract might contain from 20 to 40 per cent of a pigment which was not carotene.

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After the present work was completed, Quackenbush, Steenbock and Peterson (7) showed the presence of 5 new carotenoids in the extract from acidified alfalfa and from alfalfa silage. Three of these pigments are not differentiated from carotene by the usual methods of analysis and will cause false high values. A recent work by Wiseman, Kane, Shinn, and Cary (11) summarizes much of their work on hays and silages and states that the error from pigmented impurities varied from 11 to 32 per cent in the case of the hays and corn silage.

The question arose, therefore, whether the colorimetric determination of carotene is an accurate measure of the vitamin A potency of silages. In order to answer this question, samples of silage, representing several crops and preserved by different ensiling processes, were obtained; the carotene contents of each determined colorimetrically; and the vitamin A potency measured by biological assay. It was thought that, if some change did occur in the carotene fraction during ensiling and storage, this change might be greater in some samples than in others.

EXPERIMENTAL

In obtaining the silages, no attempt was made to sample the contents from any particular silo, but the material chosen was always of good quality and reasonably representative of that crop. All of the materials tested had been preserved in the usual tower type of silo. In each case, about 25 lbs. of silage was obtained, ground through a motor driven meat chopper, mixed thoroughly, and stored in tightly stoppered bottles in a refrigerator at 0° ± 5° C. Most of the samples showed no signs of molding or other spoilage, but in the few cases where this did occur they were immediately discarded. Carotene analyses were made at the beginning, the end, and approximately the middle of the assay period according to the method of Russell, Taylor and Chichester (8), and each sample was fed to groups of 13 to 15 vitamin A deficient rats at a level calculated to furnish 1.0 microgram of carotene per day. The assay method was essentially that recommended by the U. S. Pharmacopoeia, using the four week assay period. Although only a single level of each silage was fed, because of the larger number of animals, the average responses are believed to have more significance than would be the case with the usual group of 10 animals. Because of the small amounts of relatively coarse material which were required daily, double supplements were administered on alternate days.

The materials were stored and fed in the wet condition, since previous work had shown that a rapid destruction of carotene occurred during drying and storage in vacuo (10), whereas the carotene in wet silage was fairly stable. However, not all of the samples used in the present experiment were found to have such stability with respect to their carotene contents. It had been planned to change the feeding level to correct any change in the carotene content, but this practice did not prove feasible because of the time required in running the analyses. Therefore, the animals were fed at the original level of silage throughout the assays and the average carotene intakes were calculated from the results of the colorimetric determinations.

RESULTS AND DISCUSSION

The results of the carotene analyses are shown in Table 1. The storage periods shown represent the time which elapsed between the 1st and 2nd, and the 2nd and 3rd analyses, which cover the total period of the bioassay in each case. The final column, which shows the average carotene content, has been weighted according to the number of days which elapsed between analyses. One value, shown in parentheses, was not used in the final average because it was badly out of line and believed to be in error.

TABLE 1

Carotene content of silages

Material	Initial carotene content	First storage period	Carotene content end of first storage period	Second storage period	Carotene content end of second storage period	Average[1] carotene content
	microgm. per gm.	*days*	*microgm. per gm.*	*days*	*microgm. per gm.*	*microgm. per gm.*
Regular corn silage #1	19.3	19	16.3	22	20.3	18.1
Regular corn silage #2	9.0	27	(17.8)	22	6.4	7.7
Molasses corn silage	15.9	21	11.8	30	11.8	12.6
Molasses oats silage	10.3	27	7.6	22	6.4	8.1
Molasses soybean silage	16.8	39	9.2	8	6.6	12.1
Molasses grass silage	28.4	21	25.7	22	23.1	25.7
A.I.V. soybean corn silage	26.1	49	25.8	8	33.2	26.4
A.I.V. alfalfa silage	25.4	21	13.6	30	8.8	14.6
A.I.V. corn silage (2 years old)	7.1	......	....	51	7.1	7.1

[1] Weighted average.

Perhaps the most significant fact to be derived from these figures is that the samples varied rather widely with respect to the stability of the carotene. In certain cases the behavior appeared somewhat erratic with small losses or gains. These differences are believed to be due mainly to the difficulty of adequately sampling such heterogeneous material. In general, there was a loss of carotene during storage, this loss being most pronounced with the A.I.V. alfalfa and the molasses soybean silages. However, in no case was the loss as rapid as that which occurred when silage was dried at room temperature in a vacuum desiccator over sulfuric acid and stored in vacuo at 0° ± 5° C. The figures emphasize the fact that the vitamin content of a test material may change during the assay period so that the results may be subject to errors.

The levels of carotene present are not intended to represent the exact levels which may be expected in the crops described since no particular attempt was made to secure representative samples. It is of interest to note that one crop, corn, gave values ranging from 7.7 to 18.1 micrograms of carotene per gram of silage. It seems probable that similar variations might be found with different samples of the other crops.

The results of the bioassays are shown in Table 2. All the assays except that on the A.I.V. corn silage were run on a single large group of animals and the results should therefore be strictly comparable. The A.I.V. corn silage was not available at the same time as the other samples and had to be assayed at a later date. The molasses soybean and A.I.V. alfalfa silages, which in Table 1 showed the greatest loss of carotene, and therefore were fed at the lowest levels of carotene intake, gave low average growth responses. In this colony such a rate of growth is too low to give reliable results. Therefore, the most reliable values may be considered to be those of the regular corn Nos. 1 and 2, molasses corn, molasses oats, molasses grass, and A.I.V. soybean-corn silages.

TABLE 2

Vitamin A content of silages

Material	Daily supplement of wet silage	Average carotene content of supplement	Average growth response	Vitamin A in supplement	Vitamin A per 0.6 microgm. of carotene
	mg.	*microgm.*	*gm.*	*U.S.P. units*	*U.S.P. units*
A.I.V. corn silage (2 yrs. old	140.0	0.98	41.8 ± 2.3	3.80	2.33
A.I.V. alfalfa silage	39.4	0.56	12.7 ± 2.3	2.03	2.18
Molasses corn silage	62.7	0.79	25.4 ± 2.2	2.66	2.02
Regular corn silage #1	51.0	0.93	28.5 ± 2.9	2.85	1.84
A.I.V. soybean-corn silage	42.7	1.13	36.8 ± 2.1	3.42	1.82
Regular corn silage #2	110.0	0.86	24.2 ± 3.3	2.57	1.79
Molasses oats silage	98.0	0.79	20.4 ± 1.8	2.36	1.79
Molasses grass silage	35.2	0.90	20.5 ± 1.6	2.38	1.59
Molasses soybean silage	59.6	0.72	10.4 ± 1.9	1.90	1.58
International standard carotene	[a]	1.20	32.6 ± 2.1	3.13	1.57

[a] Fed in 0.1 ml. of corn oil.

In Table 2, the next to the last column shows the vitamin A content, in U.S.P. units, of the supplement fed. These values were obtained by reference to a growth-response-dosage curve for the U.S.P. reference cod liver oil. In order to compare the different supplements on a uniform basis, values were calculated which show the number of U.S.P. units per 0.6 microgram of carotene in the supplement. This figure was chosen as a basis for comparison since it is the value of the International Unit of vitamin A. The figures in the last column therefore show the ratios of U.S.P. units of vitamin A, as determined by biological assay, to International Units of carotene, determined colorimetrically.

An examination of these values shows, on the whole, good agreement, with the variation among the different groups probably being little more than may be expected in this type of work. If all the values are used the average is 1.88 ± 0.06 with a coefficient of variation of 13 per cent. If the 2 year old A.I.V. corn silage is not considered, since the data on this sample were obtained with a different group of rats, the average is 1.83 ± 0.05 with a coefficient of variation of 10 per cent. And if the results on the molasses soybean and A.I.V. alfalfa silages, which are considered less reliable because of the low growth response, are also dropped the values become 1.81 ± 0.04 and 7 per cent. All these averages are in close agreement and it may be concluded that one International Unit of carotene is equivalent to 1.8 U.S.P. units of vitamin A, or that 1 microgram of carotene is equivalent to 3 U.S.P. units.

The U. S. Pharmacopoeia Unit of vitamin A is considered to be biologically equivalent to the International Unit of carotene and the reference cod liver oil has been thus standardized. However, the growth responses from the feeding of the various silages, when translated into U.S.P. units by means of the growth-response-dosage curve for the reference oil, gave values ranging from 1.58 to 2.33 U.S.P. units per International Unit of carotene fed. Furthermore, a sample of the International Standard Carotene Preparation, fed at a single level, gave a growth response equivalent to 1.57 U.S.P. units per 0.6 microgram (1 International Unit) of carotene. The fact that all these supplements showed a relatively high potency in terms of U.S.P. units would seem to indicate that the carotene analyses have not given values which were too high, due to the inclusion in the carotene fraction of pigments not having provitamin A properties. These results are at variance with what might be expected on the basis of the work of Shinn and coworkers (9, 11) and of Quackenbush and associates (7). The reason is not known. It may be that the samples of reference cod liver oil used in preparing the dosage-growth-response curve were below the stated potency. To some extent the high results may be due to the natural variations which may be found to occur among groups of experimental animals. Munsell (5), for example, has reported that, over a period of 1 year, 8 groups of rats receiving identical vitamin A supplements in the form of the U.S.P. reference cod liver oil showed average growth responses in 5 weeks ranging from 16.4 to 33.5 grams. It is also possible that these differences can be explained by the work of Dyer, Key and Coward (1) and of Lathbury and Greenwood (4) which showed that different oils or even different lots of the same kind of oil, when used as diluents for vitamin A supplements, may influence the results.

The results obtained on feeding the A.I.V. corn silage are of particular interest in view of the purpose of this work. This material had remained in the silo for a period of approximately 18 months instead of the usual period of 6 to 10 months; furthermore, it was acid-treated silage. Consequently, if acid could cause the formation of pigments which would markedly raise the apparent carotene content, one might expect a correspondingly low biological value per unit of carotene fed. However, just the opposite was found to be the case. The ratio of U.S.P. to International Units, 2.33, was higher for this sample than was the case with any of the other silages, even though levels of reference cod liver oil fed at the same time as the A.I.V. corn silage gave less growth than did similar feedings made while the remainder of the assays were being run. This fact, as well as the fact that all of the other silages gave a higher growth response per unit of carotene than did the International Standard carotene, is believed to be further evidence that the formation of new carotenoids by the action of acid on green plant tissue does not interfere with the accuracy of the colorimeteric carotene determination as a measure of vitamin A potency of silages.

SUMMARY

Carotene analyses and vitamin A bioassays have been carried out on 9 samples of silage, including regular corn silage, crops ensiled with molasses, and by the A.I.V. procedure. In every case the bioassays showed a higher vitamin A potency per unit of carotene than was obtained on a sample of International Standard Carotene, but the differences were of such magnitude that the carotene analysis may be taken as a reliable index of vitamin A potency. One International Unit (0.6 microgm.) of carotene in silage was found to be equivalent to 1.8 U.S.P. units of vitamin A.

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THE VITAMIN D OF ALFALFA AND PRAIRIE HAY AND ITS UTILIZATION BY DAIRY COWS

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Several studies, such as those of Steenbock, Hart, Elvehjem, and Kletzien (12), Russell (9), Smith and Briggs (11), Bechdel, Landsburg and Hill (2), Huffman, Duncan, and Lightfoot (5), and Wallis, Palmer and Gullickson (14) have been reported in the last few years which show that most of the common roughages contain a certain amount of vitamin D. In general, these studies have been only qualitative showing particularly that the amount of vitamin D contained varies with the curing history, especially with the amount of exposure to sunshine during the curing process. They have also demonstrated that the vitamin D of roughages is an important factor in the prevention of vitamin D deficiency disturbances in calves and young stock during seasons of the year when such troubles are most likely to occur.

The importance in the human dietary of the vitamin D content of milk has been recognized for some time, but studies of the relationship of the vitamin D content of normal feeding-stuffs to the potency of the milk produced and the possibility of increasing the potency of the bulk of our country's milk supply by proper feeding and management have been overshadowed by studies of methods designed to enhance the potency to levels much higher than those obtainable by ordinary means. Hess, Light, Frey, and Gross (4), Light, Wilson, and Frey (8), and Russell, Wilcox, Waddell, and Wilson (10) have shown that when relatively large amounts of vitamin D are fed to cows in the form of special concentrates about 2 to 3 per cent is recovered in the milk. Such milk is potent enough to protect babies from rickets when fed in customary amounts. The value of such information is quite evident. The present investigation, however, includes quantitative studies of the vitamin D content of two common roughages, alfalfa hay and prairie hay, and some observations concerning its utilization by dairy cows for meeting body needs and enhancing the vitamin D content of the milk produced.

METHODS

A sufficient amount of alfalfa hay and prairie hay was obtained from local sources at one time to complete the entire experiment. Care was taken to secure hay of good, average quality and of comparable curing history. A representative sample of each lot of hay was secured at the beginning of the experiment and another one at the close to be used for a vitamin D assay.

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Six grade Holstein cows were used. All the cows had freshened in the late summer and fall and on December 15 they were placed in the experimental barn and kept out of direct sunshine until the close of the experiment. In good weather they obtained exercise after dark in a dry lot. An attempt was made to bring any excess body reserves of vitamin D to a comparable basis by feeding all animals a vitamin D-deficient ration for two months. Molasses beet pulp, which contained no measurable amount of vitamin D, was used as the only roughage material. A grain mixture to balance the ration was made up of ground yellow corn, ground oats, corn gluten meal, common salt, and a little bone meal. A vitamin A concentrate was fed to avoid a deficiency of this factor.

On February 15, after two months under the vitamin D-deficient schedule, the cows were divided into three groups of two animals each. Each animal in group 1 received twenty pounds of alfalfa hay daily in place of the molasses beet pulp and the grain mixture for this group was changed to one of equal parts of ground corn and oats. Each animal in group 2 was given twenty pounds of prairie hay in place of the beet pulp. Two animals were continued on the molasses beet pulp ration as a control group.

Just before the cows were divided into groups on February 15, a sample of butterfat was obtained from the mixed milk of all six cows for vitamin D assay to establish the potency of the milk at the time the roughage feeding started. The cream obtained from separating this milk was churned, the butter was melted and the pure butter oil filtered off, placed in a glass jar, sealed, and stored in a refrigerator until assayed. After starting the alfalfa and prairie hay feeding, butterfat samples for vitamin D assay were secured at monthly intervals from each of the three groups of cows for a period of three months.

The regular line-test technique was used in making the vitamin D assays of the hay and butterfat samples. Litter-mate rats were spread as evenly as possible over the different levels of feeding for the test materials and International Standard Solution of Irradiated Ergosterol which was used as a reference oil. The designated amount of a test material was mixed with sufficient rachitogenic diet to make a total of 40 grams. This was usually consumed in the first seven or eight days of the test period. The rachitogenic diet only was given during the remaining days of the test period.

An attempt was made to use the method of concentrating the vitamin D of butterfat reported by Bechdel and Hoppert (3) to facilitate the assaying of the less potent samples. In checking the adequacy of the method it was found that the vitamin D was not being quantitatively recovered in the alcoholic extract. The method was modified chiefly by subjecting the alcohol-fat mixture to a one hour digestion period, under a reflux condenser each time before recovering the alcoholic extract, but the results were still unsatisfactory. A brief report of these attempts is included in this paper.

RESULTS

The essential data relative to the vitamin D assays on the alfalfa hay, prairie hay, and molasses beet pulp are presented in Table 1. There was no significant difference in the healing response shown by the group of rats receiving 4.5 grams of the sample of alfalfa hay collected in the fall when the hay was put into the mow at the start of the experiment, and the group receiving 4.5 grams from the sample collected near the end of the experiment. Likewise, the healing by these two groups of rats on the test material is essentially the same as that shown by the Standard Reference group receiving 5 International units of vitamin D. On this basis 1 gram of the alfalfa hay contained 1.10 International units of vitamin D. The 12 rats receiving 9 grams of the sample of prairie hay collected near the end of the experiment showed practically the same healing as 12 litter mates that received 5 International units of vitamin D, indicating a potency of 0.55 International units per gram of prairie hay. The smaller group of rats used on the prairie hay sample collected in the fall shows slightly less healing but this is more likely due to differences in individual responses for this smaller group of rats, some of which were not litter mates, than to any significant change in the potency of the hay. Vitamin D could not be detected in the molasses beet pulp even when it was tested at a 15 gram level.

TABLE 1

The vitamin D content of alfalfa hay, prairie hay, and molasses beet pulp

Material	Number of rats	Average healing*	Vitamin D per gram
			*I.U.***
Alfalfa hay			1.10
4.5 gm. sampled at beginning	8	0.43 +	
4.5 " " " end	11	0.55 +	
5 I.U.—Standard Reference Oil	11	0.57 +	
Prairie hay			0.55
9 gm. sampled at beginning	7	0.70 +	
9 " " " end	12	0.90 +	
5 I.U.—Standard Reference Oil	12	0.96 +	
Molasses beet pulp			.. ....
15 gm.	12	0.00	

* A narrow continuous line of mineral deposition is evaluated as a 1.0 + healing.
** I.U. = International unit of vitamin D.

The results of the vitamin D assays on the butterfat samples are shown in Table 2. The butterfat produced by the cows in group 1 showed a steady increase in potency from 0.19 International units per gram at the start to 0.46 International units at the end of three months. These cows received 10,000 International units of vitamin D daily in the 20 pounds of alfalfa hay fed. The butterfat produced by group 2 receiving 5000 International

TABLE 2

The effect of the vitamin D ingested from alfalfa hay and prairie hay on the potency of the butterfat produced by dairy cows

Group	Vitamin D fed per cow daily	Vitamin D per gram of butterfat			
		At start of experiment	After experimental feeding for		
			1 month	2 months	3 months
	*I.U.**	*I.U.*	*I.U.*	*I.U.*	*I.U.*
Group 1 Alfalfa hay	10,000	.19	.21	.32	.46
Group 2 Prairie hay	5,000	.19	.15	.16	.22
Group 3 Beet pulp		.19	Too weak to measure		

* I.U. = International unit of vitamin D.

units of vitamin D in 20 pounds of prairie hay declined from 0.19 International units per gram of butterfat at the start to 0.15 International units for the first month then increased slowly to 0.22 International units per gram at the end of the three months' period. The butterfat from the beet pulp control group decreased in potency until it was too weak to measure, although there was some evidence of a trace of vitamin D in some of these samples.

The results of studies on methods for concentrating the vitamin D of butterfat to facilitate the assay of relatively impotent samples are summarized in Table 3. Method 1 refers to the procedure outlined by Bechtel and Hoppert (3). In trial 1 nine rats receiving 2.4 grams of summer butterfat fed directly at the rate of 0.3 gram daily for the first eight days of

TABLE 3

The effectiveness of methods of concentrating the vitamin D of butterfat for assay purposes

Material tested	No. of rats	Average healing*
Method 1, Trial 1		
2.4 gm. fat—direct	9	0.75 +
Extract from 2.4 gm. fat	9	0.03 +
Reference Standard—4 I.U.**	4	1.31 +
Method 1, Trial 2		
Extract from 5 gm. fat	6	0.79 +
Residue from 5 gm. fat	5	0.38 +
Method 2, Trial 1		
3.2 gm. of fat—direct	8	0.91 +
Extract from 3.2 gm. of fat	9	0.33 +
Residue from 10.6 gm. of fat	3	1.16 +

* A narrow continuous line of mineral deposition is designated as a 1 + healing.
** I.U. = International unit of vitamin D.

the test period showed an average healing of 0.75 +. Nine litter mates receiving extract equivalent to 2.4 grams of the same fat showed an average healing of only 0.03 +. The extract was mixed with a 40-gram portion of rachitogenic diet and fed during the first part of the test period. The sensitivity of the colony at this time is indicated by the 1.31 + healing shown by four rats receiving 4 International units of vitamin D in Standard Reference Oil.

In trial 2 each of six rats was fed an amount of extract equivalent to 5 grams of fat. Litter mates were fed the residue left from the extraction process in an amount equivalent to 5 grams of the original fat to see if unrecovered vitamin D could still be detected in the fatty residue. The extract equivalent to 5 grams of fat showed an average healing of 0.79 + and the fatty residue gave a healing of 0.38 +, which furnishes direct evidence that all the vitamin D was not being recovered in the extraction process.

The procedure was modified to include a digestion of the alcohol-fat mixture under a reflux condenser for a period of one hour for each of the successive extractions made on a butterfat sample. The results of this modification are shown under Method 2 in Table 3. Eight rats receiving 3.2 grams of fat directly showed an average healing of 0.91 + while nine litter mates receiving the extract equivalent to 3.2 grams of the same fat showed healing of only 0.33 +. The fatty residue left from extracting the 32 grams of fat was divided into three equal parts so that each portion represented the fatty residue left from the extraction of 10.6 grams of fat. The rats receiving these portions showed an average healing of 1.16 +, indicating that an appreciable amount of vitamin D still remained in the fatty residue. These results vary widely from the experiences reported by Bechtel and Hoppert (3), but are in close agreement with the extensive studies of the English investigators, Kon and Booth (6) (7). As there seemed to be no method available which could be used with confidence for quantitatively concentrating the vitamin D of butterfat, the samples were assayed as completely as possible by feeding the butterfat directly.

DISCUSSION

The alfalfa hay used in this investigation showed twice the vitamin D potency of the prairie hay, although both lots of hay were of good average quality and had comparable curing histories. Whether alfalfa will in general be found to contain more vitamin D than prairie hay cannot be determined until more samples have been tested. The vitamin D found in these samples amounts to 500 International units per pound of alfalfa hay and 250 units for prairie hay. The significance of these figures is emphasized when they are compared with the vitamin D requirements of animals. For instance, Bechdel, Hilston, Guerrant and Dutcher (1) have shown that about 300 International units of vitamin D daily per 100 pounds of liveweight will suffice to protect a calf from rickets up to seven months of age. Two pounds of the alfalfa hay used in this experiment would meet the above requirements for a seven-months-old calf. These observations are in harmony with experimental results wherein calves have been protected from a deficiency of vitamin D by small amounts of hay.

It has been shown by experimental work at the South Dakota Station, Wallis (13), that liberal-milking cows have a certain vitamin D requirement which must be met if severe deficiency symptoms are to be avoided. The alfalfa and prairie hay used in this experiment evidently supplied enough vitamin D to meet the body requirements of the cows and to furnish an available surplus which provided for an increasing amount of vitamin D in the butterfat produced. Calculations based on the fat percentage and the amount of milk produced during the third month of the experiment show that 1.77 per cent of the 10,000 International units of vitamin D in the alfalfa hay was recovered in the milk. Similar calculations indicate that 1 per cent of the 5,000 units being fed the cows on prairie hay was recovered in the milk. Just what effect the amount of milk produced and the fat percentage of the milk may have on the amount of vitamin D recovered from a given intake cannot be definitely stated, but variations in these factors would undoubtedly affect the results obtained. The higher percentage recovered in the milk from the alfalfa hay group may be due to the fact that one of these cows was still milking much more liberally than the rest of the animals at the time the samples were taken for the third month. In any event, the amount of vitamin D recovered in the milk represents only a small percentage of what is ingested in the normal rations of dairy cows. The percentage of vitamin D recovered from the smaller amounts in normal rations is of about the same order as the percentage recovered from the feeding of larger amounts in the form of vitamin D concentrates as previously mentioned in the introduction.

The amount of vitamin D in the butterfat from the cows on the molasses beet pulp decreased to such an extent that it was no longer possible to measure it, although occasionally a rat receiving 12 or 15 grams of this fat would show a slight amount of healing. Subsequent work at this station, Wallis (13), has shown that liberal-milking cows may deplete themselves of vitamin D in three or four months. Butterfat from such animals will not initiate healing in rats when fed at 12 to 15 gram levels. The slower decrease in the vitamin D potency of the butterfat from the beet pulp control group in this experiment may be due to the smaller milk production of these animals as they were nearing the end of their lactation. In fact, one cow in this group went dry before the experiment was completed.

The results of this experiment indicate that even with the smaller vitamin D intake of normal rations there is a tendency for the vitamin D in the butterfat to increase with increases in the vitamin D content of the ration.

Thus, at the end of three months the butterfat from the group getting 10,000 units daily in alfalfa hay showed a potency of 0.46 International units per gram, that from the prairie hay group getting 5,000 units daily showed a potency of 0.22 International units per gram, and that from the beet pulp control group was too weak to measure. These results indicate that the vitamin D of hay constitutes at least one of the sources of the vitamin D which appears in the milk. Although the absolute potencies indicated for the less potent samples of butterfat should not be emphasized too strongly because of the difficulties involved in accurately assaying such small amounts of vitamin D, the general relationships just pointed out are fairly clear cut and are undoubtedly significant.

SUMMARY

Representative samples from comparable lots of alfalfa hay and prairie hay were assayed for vitamin D. The alfalfa contained 1.10 International units, and the prairie hay 0.55 International units of vitamin D per gram. When these lots of hay were fed to comparable groups of dairy cows the butterfat produced by the group receiving alfalfa hay was more potent in vitamin D than the butterfat from the group receiving prairie hay, which was in turn more potent than butterfat from a beet pulp control group. Between 1 and 2 per cent of the vitamin D in these normal rations was recovered in the milk. Attempts to quantitatively concentrate the vitamin D of butterfat to assist in the assay of relatively impotent samples were unsuccessful.

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HIGH RECORDS CONTRASTED WITH UNSELECTED RECORDS AND WITH AVERAGE RECORDS AS A BASIS FOR SELECTING COWS*

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While making some preliminary studies of suitable ways to test the relative values of production records, singly and in various combinations, for predicting future records of the same cow and for evaluating her transmitting ability, we noted an article by Copeland (2) purporting to show some advantages for using a cow's highest record as an index to her lifetime producing ability and to her transmitting ability. As our data, which came from a different source, seemed appropriate for testing Copeland's conclusions, we repeated a part of his analysis on our own material and added a few other features which, it is hoped, will demonstrate why the *ex post facto* selection of the highest record is an unsound procedure—a procedure more likely to lead to fallacious results than if an unselected record or, better still, the average of all a cow's records were used as the measure of her ability.

A major difficulty associated with the use of the highest record in comparing cows (and not mentioned by Copeland) is that in practice most comparisons will need to be made between cows which have not completed an equal number of recorded lactations at the time the comparison must be made and which, therefore, have not had the same number of chances to make a high record. The obvious unfairness so introduced seems of so much practical importance that we will refer to it again in some detail after first presenting our findings on a basis comparable with Copeland's.

DATA STUDIED

We studied the records of 115 Holstein cows, each of which had completed at least six Herd Improvement Registry lactations and had one or more daughters each of which had completed at least two records.[1] All records were brought to a mature "B" basis by use of the conversion factors listed in the Holstein Red Books.

FINDINGS

The first step was to compare the correlations between various records and averages. These are shown in Table 1 along with the corresponding ones

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[1] These data were those included in some originally assembled for another purpose—that of proving sires. Here a 6-record cow was used as many times as she had tested daughters. Actually there were 89 different cows. If the dam of a 6-record cow had two or more records, that dam was treated as a daughter. The relation is a parent-offspring one in either case. The findings should be practically the same as if there had actually been 115 different 6-record cows, each with one tested daughter.

reported by Copeland. For the four "repeatability" comparisons (the first four in Table 1), our coefficients are lower than those reported by most students of this question, while those of Copeland are among the highest we have ever seen. More typical are findings like those of Plum (5) that a correlation of .60 existed between records of the same cow in the whole population of records from many herds, but that on an intra-herd basis the correlation between records of the same cow was reduced to .40. Perhaps a high degree of heterogeneity from herd to herd existed in Copeland's data.

TABLE 1

Correlation coefficients for Holstein H.I.R. records, Jersey R. of M. records and Jersey Herd Test records

Variates correlated	Coefficients of correlation		
	115 Holstein H.I.R. cows	197 Jersey R. of M. cows*	166 Jersey Herd Test cows*
First record with the second	.30	.71	.78
Second record with the third	.42	.77	.80
Third record with the fourth	.55	.69	.75
Fourth record with the fifth	.45	.59	.83
First with ave. of next four	.49	.62	.80
First with ave. of all five	.68	.75	.88
Highest with ave. of all five	.90	.92	.92
Highest with ave. of other four	.83	......	.....
Lowest with ave. of all five	.79	......	......
Lowest with ave. of other four	.70		

* Copeland's data.

If the various records were all equally correlated (r) with each other, the correlation between the first and the average of the next four would be expected to be $r\sqrt{\frac{4}{1+3r}}$. It is a little lower than this (if for r is used the simple arithmetic average of the four repeatability correlations shown) in all three sets of data (especially so in the Jersey R. of M. records), thus indicating that the correlations between non-consecutive records (not shown) average somewhat lower than those between consecutive records. Similarly, if all the correlations between the individual records were equal to r, the correlation between the first record and the average of the first five records would equal $\sqrt{\frac{1+4r}{5}}$. Again the actual figures are lower (much lower in the case of the Jersey R. of M. records) than they would be if the non-consecutive correlations averaged as high as the consecutive ones which are shown.

The numerical values to be expected in the case of the correlation between *the highest record* and the average of all five are not clear because of the complex statistical relations brought about by the *ex post facto* selection of the highest record. That such selection has an effect on the correlation subsequently calculated can be made clear by considering an extreme case in which it is assumed that we have samples of five records each from cows between which there really are no intrinsic differences, the variation between the various records being wholly due to varying external circumstances and to sampling variations. This is the same statistical problem as would exist in studying random samples of five records each, drawn from the same universe. Since all of the other four records must be lower than the high record, it follows that the samples in which the high record itself has a low value will be samples in which all five records happened to come from the lower part of the frequency curve. But in such cases the other four will rarely be *much* below the high one and the two variates will be closely correlated.

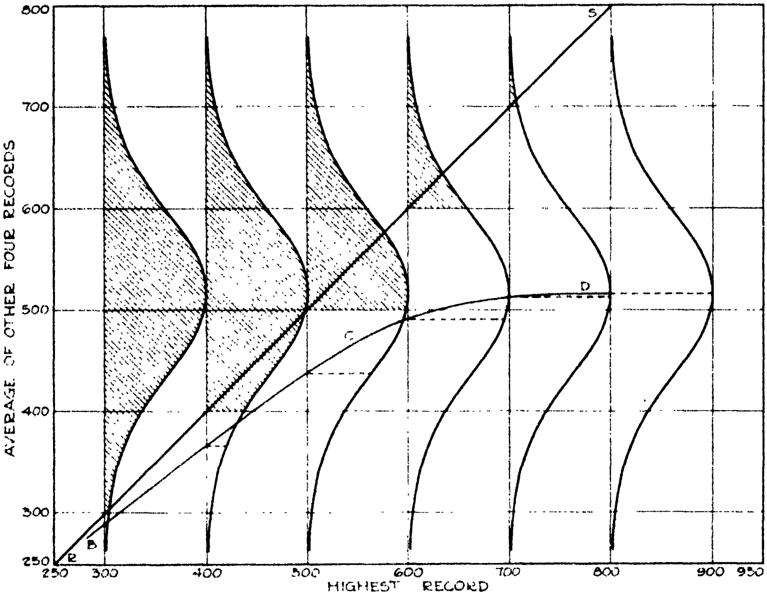

FIG. 1. The statistical consequences of the *ex post facto* selection of the highest record among five. The normal curves, all drawn at the same height on the ordinate axis, illustrate the variation to be expected between different records of the same cow in a population of cows which all have exactly the same real ability. In such a population it would sometimes happen that the highest of five records would be very high; sometimes it would be moderately high; and sometimes even the highest record would be moderately low. When a sample of five records is located along the abscissa according to the highest record in it, the other four must come from the unshaded portions of the frequency curves —*i.e.*, from below the line RS. When the highest record is large, it can be considerably larger than the average of the other four records; but when the highest record is itself rather low, it cannot be very different from the average of the other four. This gives rise to a high correlation between the high and the average of the others, to curvilinear regression, and to a funnel-shaped distribution.

Figure 1 is intended to show graphically what would happen in such a case if the highest of the five records in each sample were correlated with the average of the other four. Six normal curves with their means at the same level have been drawn at intervals of 100 lbs. along the abscissa to show the restrictive effect of locating the sample along the abscissa according to the highest of the five records in it. In each case the other four records must all be below the diagonal RS, and must come from the unshaded portions of the normal curves. At the right hand side of the graph (where the high record happens to be very high) little restriction is laid on the magnitude of the other four records, but toward the left hand side most of the unshaded portion of the curve lies very near the diagonal RS and the average of the other four records cannot be far below the high record.

Because of the constricting influence of the high record on the magnitude of the other four, the total distribution becomes funnel shaped and the line BCD (drawn through the means of the unshaded portions of the six normal curves) showing the regression of the average of the other four on the high one is curvilinear in character. The extreme narrowing of the distribution, when the high record itself is not very large, is particularly potent in giving a large correlation between the high record in the sample and the average of

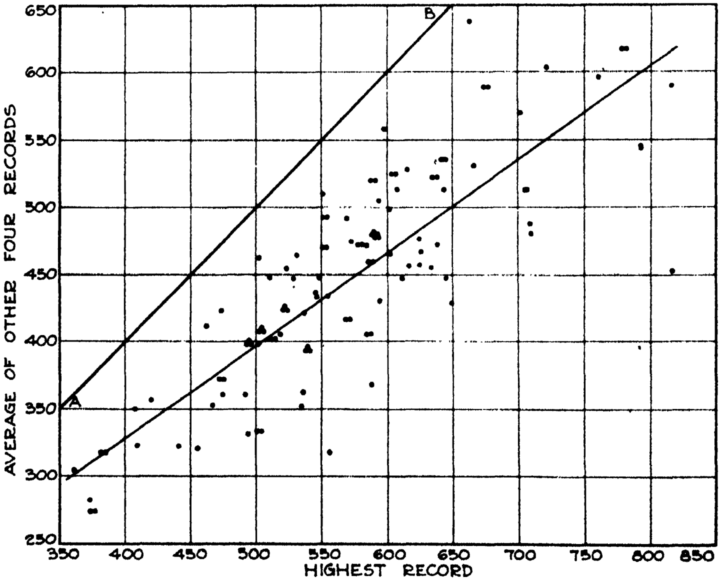

FIG. 2. Correlation (.83) between the highest record and the average of the other four records in a population of 115 H.I.R. cows. All points must fall below the diagonal AB. The regression line is the best-fitting straight line but does not tell the full story, since the real relation between the two variates is curvilinear. The restrictive influence of the highest record on the magnitude of the other four records is especially evident toward the left side of the diagram.

the other four records, even in an extreme case like this where the samples come from the same universe (as from a group of cows all with the same producing ability).

Actually, of course, there are differences between the cows, too, and this gives the regression line more of an upward slope and somewhat less curvilinearity although the funnel shape of the data and the extreme restriction at the left are conspicuous features of the actual data as shown in Figure 2. It is thus apparent that the correlation coefficient for the high record and the average of the other four owes a part of its magnitude merely to the fact that the first mentioned variate was selected *because* it was the high record. Because of the curvilinearity and the funnel shape, the biometric relations seem complex and we do not know how to separate the observed correlation coefficient into the spurious part which comes from this useless *ex post facto* arrangement of the data and the part which would show the genuine usefulness of the high record in predicting records not yet made when the high one was selected.

When the highest record is correlated with an average of the five which include it, the coefficient becomes still larger. The situation is the same as shown in Figure 2 except that each point is now moved upward one-fifth of the distance which in Figure 2 separates it from the line AB. Naturally those lying farthest away are moved most and so the correlation is distinctly increased but no new knowledge is gained.

Figure 3 shows the first record as correlated with the average of the other

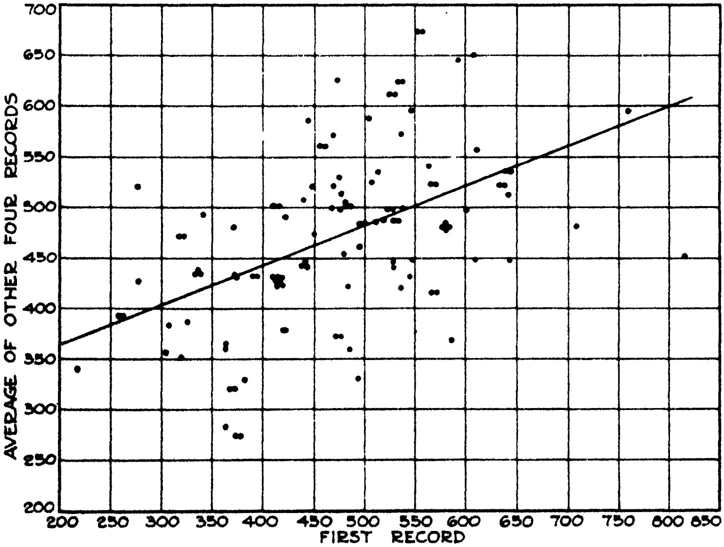

Fig. 3. Correlation (.49) between the first record and the average of the next four records. Same cows as in Figure 2. The straight line shows the linear regression of the average of the other four records on the first record.

four. Using the first record sets no restriction on the magnitude of the average of the other four. The other four can all be below the first, or all above it, or some on one side and some on the other. Such a correlation is an unbiased estimate of the usefulness of the first record for predicting the average of the other four.

WHY NOT THE LOW?

To demonstrate more clearly how subsequent selection of a record *because of* its size affects the correlation coefficient, the lowest of the five records was correlated with the average of the remaining four and also with the average of all five. In our data the low record was less variable ($\sigma = 82$) than the high ($\sigma = 96$) and hence had less influence in determining the variation of the averages of five. For that reason alone, the low might be expected to be somewhat less closely correlated with the average than was the highest record. Moreover, the forces which tend to make the highest record large and those which tend to make the lowest record small may be different enough that the corresponding covariances may not be entirely comparable. For example, some of the low records may have been made under circumstances definitely known to have been abnormal but not specific enough that it would be practical for us (or for the breed association) to undertake any corrections for these circumstances. (Although this is plausible, it seems not to have been important, because the low records averaged only 102 pounds below the average of all five, whereas the high records averaged 98 pounds above. Therefore the distribution seems to have been nearly symmetrical.) While the coefficients for the lowest record (.79 and .70 in Table 1) are not as large as those for the highest record, yet they are much larger than correlations involving unselected records. The reasons are the same in principle as those discussed in connection with Figures 1 and 2.

Before passing from this consideration of the lowest record some comment might be ventured relative to the practical considerations involved. Dairymen frequently raise objections to including all records of production in averages because they feel that some lactations are abnormal. Such objections are usually raised, however, *after* the cow has completed (or nearly completed) a low record. That is, it is difficult (in practice usually impossible) to know whether the circumstances were judged abnormal solely because of them or largely because it was seen that the record was going to be low. Theoretically, the omission of records known to have been made under definitely abnormal conditions for which adequate corrections cannot be made would, we think, enhance the value of the resultant averages. However, practical difficulties connected with deciding what constitutes grounds for omission, and with making sure that the size of the record itself does not influence the decision to use it or omit it, render the advisability of omitting any records from lifetime averages problematical. Certainly the reasons for such omission would have to be so specific and important that the decision in each case would be automatic. For example, in Denmark only those records made in years when the cow aborted or when she had foot-and-mouth disease are omitted from her average.

THE PREDICTION OF FUTURE RECORDS OF THE SAME COW

The utility of the high record, as compared with that of unselected records, may be tested in the following way freed (we think) of those statistical fallacies which beset the correlation of the high record with the others *from which* it was selected. The highest, lowest and medium records among the first three were compared with the fourth and with the fifth; and then the unselected first, second and third were correlated also with the fourth and fifth records. The results of such a fair test on our data are shown in Table 2. The differences between the correlations in the same column are not statistically significant (on this amount of data—probably the question should be investigated on more extensive data), but the contrast with the figures in Table 1 shows clearly that much of the apparent superiority of the high record (and of the low) in that table was due to the spurious effects of the method of its choice.

TABLE 2

Correlation coefficients between a cow's first three records (selected and unselected) and her later records

The six variates correlated with the fourth and fifth records	Correlation coefficients with	
	Fourth record	Fifth record
Highest of first three	.61	.29
Lowest of first three	.56	.45
Medium of first three	.57	.31
First record	.39	.39
Second record	.56	.24
Third record	.55	.26

THE PREDICTION OF DAUGHTER'S RECORDS

Table 3 shows the correlations of the average of the first two records of the daughters with the high and low of all five records, with the high, low and medium of the first three, and with the five unselected single records. For comparison, Copeland's coefficients are included in this table. All the coefficients are below .34. The considerable differences between some of them do not seem to fit any particular pattern and are not statistically significant on this amount of data. So far as these data go, any one of these records is about as useful as any other for predicting the production of daughters.

THE USE OF AVERAGES

Averages of all available records are theoretically more useful than single records in evaluating the differences between cows. The authors are at present studying, on a larger amount of data, the question of whether records actually do behave in this way. Some of the preliminary results are presented in Tables 4 and 5.

TABLE 3

Correlation coefficients between the average of the first two daughter records and various records of the dam

Dam's record which was correlated with the average of the daughter's first two records	Correlation coefficients
Highest of all five records	.29
Lowest of all five records	.15
Highest of first three records	.24
Lowest of first three records	.19
Medium of first three records	.33
First record	.24
Second record	.14
Third record	.23
Fourth record	.29
Fifth record	.13
Copeland's comparisons	
Highest records of 176 dams and highest records of the daughters	.29
Average records of 176 dams and average yield of their tested daughters	.30

TABLE 4

Correlations of averages of a cow's first records with her subsequent records and with her daughter's records

Dam's average	Correlation coefficients					
	Dam's fourth record		Dam's fifth record		Daughter's ave. of first two	
	Actual	Exp.	Actual	Exp.	Actual	Exp.
Ave. of first two records	.59	.47	.38	.47	.24	.25
Ave. of first three records	.65	.51	.38	.51	.27	.27
Ave. of first four records	....	....	.44	.53	.30	.29–
Ave. of all five records	...	....	..	.	.29	.29+

TABLE 5

Correlation coefficients between the average of the last three records of six-record cows and the first three records taken singly and in various combinations

Records correlated with the average of the last three records	Correlation coefficients	
	Actual	Expected
First record	.42	.49
Second record	.45	
Third record	.46	
Average of first and second	.54	.59
Average of first and third	.53	
Average of second and third	.54	
Average of first, second and third	.58	.64

On comparing these tables with Tables 1 to 3, it seems that there is a trend toward increased prediction value of average records as compared with single records.

The "expected" values included in Tables 4 and 5 are the ones which would have occurred if the various records of the same cow had all been equally correlated with each other and if they all had had equal standard deviations and if they had all been equally correlated with the daughter average. The correlation coefficients used in calculating the expected values in Table 4 were the arithmetic means of the ten intercorrelations between the first five records (.39) and of the five coefficients between the cow's five records and her daughter's average (.21). In Table 5, the inclusion of the sixth record gave a mean repeatability of .37.

The divergence of observed from expected values presumably was caused by actual inequalities among those various correlations and standard deviations and by the limited amount of data studied. The expected values, however, serve to indicate the magnitude of the increase which, theoretically, should occur as the correlation coefficients are based on averages of larger and larger numbers. Probably the results will become more regular when more data are included. The present amount of data is not large enough to be expected to show significant differences.

TABLE 6

Distribution of the highest record of five over the five lactation classes

Lactations	First record	Second record	Third record	Fourth record	Fifth record
Number of high records	27	24	20	23	21

THE CASE OF COWS WITH UNEQUAL NUMBERS OF RECORDS

Table 6 shows that the highest record made by a cow is about as likely to occur in any one of the first five lactations as in any other. Assuming a perfectly even distribution of the high record, it follows that the probability that a cow with just one record has already made what would be the highest in five if and when she completed the additional four is .20; in the case of a cow with two records the probability is .40; for the three-record cow it is .60; for the four-record cow, .80; and for the cow with five records, the probability is, of course, 1.0. This shows clearly the unfairness of using the highest record as a basis for comparing cows which have completed unequal numbers of lactations.

The bias introduced by unequal numbers is evident also upon considering the ratio, range/σ. The standard deviation (σ) of records of the same cow, as calculated from our data, is 81.6 pounds. Using Tippett's (6) values for range/σ for varying values of n (the number of records) it is determined that with a cow like the average of ours when $n = 2$ the high record is expected to be 46.0 pounds of butter fat above the mean (473.0 pounds) of the whole sample of 575 records. When $n = 3$ the high record is expected to be 69.0

pounds above the mean; when $n = 4$, 84.0 pounds above; and when $n = 5$, 95.0 pounds above. In other words, the expected value for the highest record of two is $473 + 46 = 519$ pounds; of three, 542; of four, 557; and of five, 568 pounds. The actual values for the average of the highest of the first three and of all five were calculated for the 115 cows of this study. They proved to be 545 and 571 pounds respectively—values that agree well with those expected theoretically. The bias of comparing the highest records only, when cows have different numbers of records, is obvious.

Copeland thinks it impractical to compare lifetime records of daughters and dams in proving bulls because the daughters and many of the dams will not have completed their lifetime of production at the time when, by using single records or by using only the highest records, it becomes possible to "prove" the bull. In using lifetime records for proving bulls, one merely uses all the information available at the moment when a decision has to be made. In using all of a cow's completed records as her average it is no more necessary to wait until her lifetime is ended than it is necessary, when proving a bull, to wait until records have been made by all of the daughters he will ever sire. The greater the number of animals and the larger the number of records completed by them, the less the error likely to be involved in the "proof." That the figures for the proof next year or the year afterward may be a little different usually means only that the later figures, because of the additional records included, are a bit more dependable. Breeders of dairy cattle are continually faced with the necessity of making decisions concerning cows which have completed different numbers of lactations. The most dependable procedure is to use all the lactation records available at the time the decision needs to be made.

In comparing cows which have completed unequal numbers of records and which have been selected for comparison because their averages are high (as in choosing a son of a high producer), or because their averages are low (as when deciding which of two cows to cull), it is necessary to take account of the decreased variability of averages based on large numbers. Otherwise it would almost always appear that the best producers—and also the worst producers—were cows with only one or two records. The fairest way of doing that seems to be to estimate each cow's real productive ability as equal to the herd average plus (or minus) $\frac{nr}{1 + (n - 1)r}$ times the excess (or deficit) of her average over the herd average.* In this formula n is the number of lactations included in her average and r is the repeatability of records of the same cow—a figure which is usually around $+.3$ to $+.4$ on an intra-herd basis. When it is .4 the above fraction reduces to the simple form $\frac{2n}{3 + 2n}$. In its use a cow with one record is credited with 2/5 of her apparent superiority, one with two records is credited with 4/7 of the apparent superiority of her average, etc. (Similarly in case her average is below the herd average, her real productivity would be estimated at 2/5, 4/7, etc., as far below the herd average as her own actual average is.) This makes it possible to compare, without bias from differences in the number of records they have completed, cows which were selected because their records were high or because they were low. No such precaution is needed in proving sires because their daughters must be unselected (if the proof is to have much validity) and the dams will not often have been selected intensively enough for such a discounting to be important. Usually there will be both low and high producers among the dams (and among the daughters) and the errors from not discounting those with high records will tend to cancel the opposite errors from not discounting those with low records.

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* For three independent derivations of this formula, each from a slightly different point of view, see references (1), (3) and (4).

SUMMARY

The high correlation between a cow's highest record (or her lowest) and the average of the other records from which this one was selected results largely from the statistical effects of this selection itself. This high correlation does not indicate superiority of the selected record for predicting future records or breeding value. When the highest record is correlated with other records from which it was *not* selected the resulting coefficient (provided all cows have the same number of records) indicates that the high record is of somewhere near the same reliability as an unselected record but almost certainly less reliable than the average of all unselected records. Differences in number of completed records, however, is of so much practical importance in making selected records unfair that the use of the highest record, as an indication of a cow's lifetime producing ability, cannot be recommended.

Averages appear to be more dependable than either selected or unselected single records for evaluating differences between cows.

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GRAPHIC ESTIMATION OF MILK PROTEIN AND MILK ENERGY

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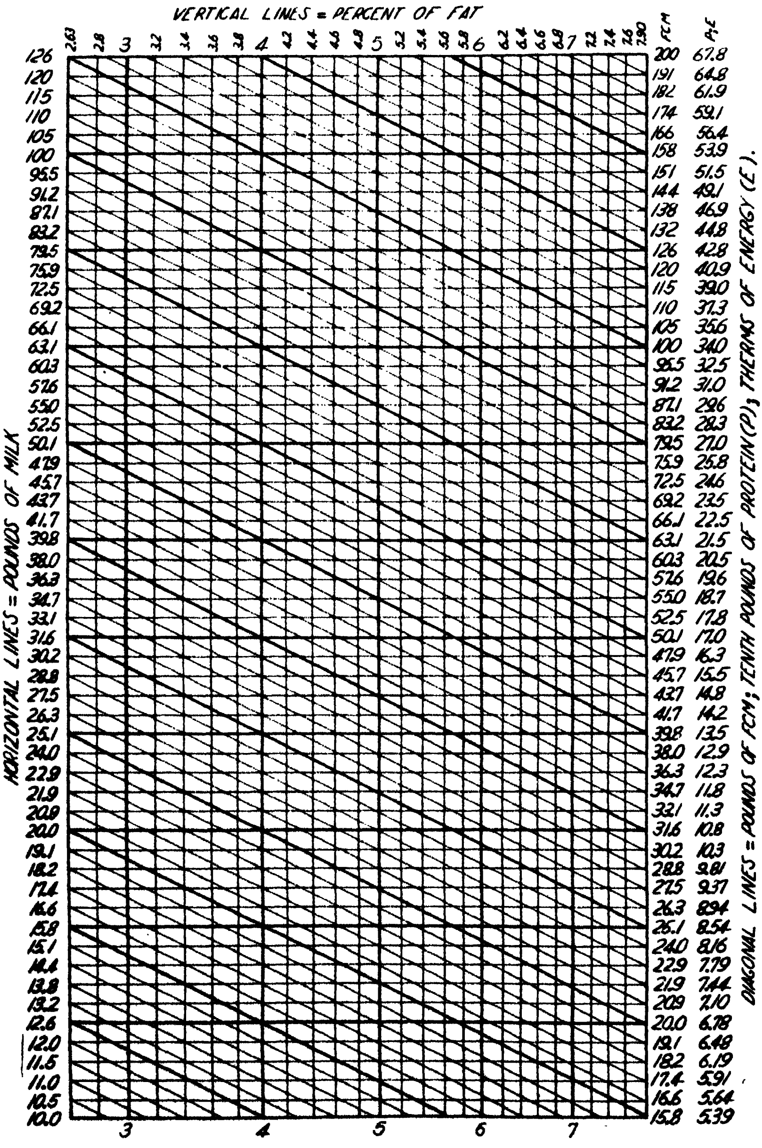

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Several designs have been used in the graphic application of the formula, $E = M(.4 + .15f)K$, in which E is milk energy, M is weight of milk, f is per cent of fat in the milk, and K is a constant (1). Milk energy is approximately proportional to milk protein (2). Accordingly, by adjusting the value of K, the right-hand member of the above equation gives the amount of protein or the amount of energy, either in absolute units or in terms of milk of a given fat percentage. The chart on the preceding page is designed to estimate, for a certain weight of milk of a certain fat percentage, the amount of protein and the amount of energy in the milk, first in terms of normal whole 4 per cent milk (FCM), second in absolute units. It is based on the relations, $FCM = M(.4 + .15f)$ and 100 pounds FCM = 3.4 pounds protein = 34 therms milk energy.

An example will illustrate the use of the chart. Find the intersection of the 30.2 pounds milk horizontal with the 7.4 per cent fat vertical. Follow the closest diagonal to the right margin and read, 45.7, 15.5. Thus, according to the chart, 30.2 pounds of 7.4 per cent milk contain 1.55 pounds of protein and 15.5 therms of energy; which is the same amount of protein and the same amount of energy contained in 45.7 pounds of 4 per cent milk (FCM). Follow the same diagonal to the intersection with the 5.4 per cent fat vertical, thence follow the closest horizontal to the left margin and read 38.0, that is 38.0 pounds of 5.4 per cent milk likewise contain 1.55 pounds of protein and 15.5 therms of energy. In other words, a given diagonal cuts the lines for milk and fat per cent at equal protein and energy content. As between breeds there may be a small systematic error in the protein estimate, the formula tending to over-estimate slightly for Guernsey and Jersey cows, and under-estimate slightly for Holstein cows.

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THE FLUORINE CONTENT OF VARIOUS FRACTIONS OF MILK AND COMMERCIAL CASEINS

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Analysis of the various constituents of a low fluorine diet in a recent series of experiments indicated that certain commercial caseins were badly contaminated with fluorine. On the average, fresh milk contains 0.1–0.28 parts of fluorine per million. On the basis of these figures one would expect at most only 10 p.p.m. in the casein if the total milk fluorine were concentrated in the product. Commercial casein purified in our laboratory by repeated reprecipitation results in a casein low in fluorine. A sample of casein used was found to contain more than 100 p.p.m., and this could be reduced to 22 p.p.m. by washing several times with dilute acetic acid. This observation led us to make a systematic survey of the fluorine content of the various fractions of milk and of commercial casein.

In these studies all of the fluorine analyses were made by the method previously described (1).

A commercial whole milk was analyzed for its fluorine content. Casein, heat coagulable protein, ether extract, and residue were separated from the milk and each constituent analyzed for its fluorine content. The whole milk contained 0.204 p.p.m., while its constituent parts contained respectively: casein, 0.053 p.p.m.; heat coagulable protein, 0.012 p.p.m.; ether extract, 0.012 p.p.m.; and the residue, 0.145 p.p.m.

The analyses of the various samples of crude casein are given in Table 1.

TABLE 1
The fluorine content of commercial casein

Shipment received	Fluorine content
	p.p.m.
2/1/38	198
3/15/38	115
4/28/38	17
7/1/38	14
8/25/38	359
8/25/38	319
10/25/38	16
Labco	4
Reprecipitated crude casein	4–5

It is apparent that these caseins were contaminated with fluorine. Certain samples were sufficiently contaminated as to cause concern where such products are to be used for nutritional purposes. A casein which contains

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300 p.p.m. of fluorine would be sufficient to introduce 50–70 p.p.m. where casein supplied the main part of the protein of the ration. This level would cause a very marked increase in the skeletal fluoride as indicated by the work of Ellis and Maynard (2) and unpublished data from this laboratory.

SUMMARY

Whole milk contains small quantities of fluorine. The fluorine content of milk is not concentrated in the protein fraction but can be found largely in the aqueous phase. Therefore uncontaminated caseins should be relatively low in fluorine. Certain commercial crude caseins were found to contain unexpectedly large amounts of fluorine. The advisability of using such contaminated crude caseins for nutritional purposes is questionable in the light of previous experience.

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FACTORS TO BE CONSIDERED IN SELECTING CHOCOLATE-FLAVORED MILK* †

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INTRODUCTION

Very few people question the healthfulness of plain milk but many question the desirability and healthfulness of chocolate milk especially for children. The extensive use of chocolate milk by children has been criticized for the following reasons:

1. Cocoa and chocolate contain theobromine and caffeine.
2. Chocolate milk has a higher sugar content than plain milk.
3. Food value of cocoa is negligible for the amounts used in chocolate milk.
4. Cocoa and chocolate contain tannin-like substances.
5. Cocoa may decrease the digestibility of the milk.
6. In general there are no bacteriological standards for chocolate milk as there are for plain milk, thus giving very little protection to consumers of chocolate milk.
7. Skim milk or low fat milk is sometimes used instead of a milk base meeting state and municipal requirements for fat and solids content.

In spite of these adverse criticisms to the extensive use of chocolate milk by children, the sales of chocolate milk have been increasing. The addition of chocolate or cocoa to milk increases the consumption of milk, which no doubt offsets some of the criticisms. The Milk Dealer (1), July, 1935, in reporting a nation-wide chocolate milk survey mentions that eighty per cent of the chocolate milk sales volume is "plus" volume and not "replacement" volume. This fact shows that, in general, chocolate milk consumers are not regular milk drinkers, and indicates that the addition of chocolate syrup to milk increases the palatability of the milk for many consumers, thus increasing the consumption.

From the foregoing it is quite evident that mothers, and those who have charge of school cafeterias, might be confused in making a choice between

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* As used in this paper the terms "chocolate flavored milk" and "chocolate milk" unless otherwise specified, will include chocolate milk made from a whole milk base and also "chocolate flavored dairy drinks." This latter term is commonly understood to mean in many localities a chocolate flavored drink made from skimmed or partially skimmed milk.

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plain milk and chocolate milk. If the choice is chocolate milk then the problem of selecting the proper cocoa or chocolate-flavored syrup must be solved. This is a difficult task because, generally speaking, there are no standards for chocolate milk drinks as there are for plain milk. For this reason commercial chocolate milk drinks vary widely in composition.

The question arises as to what factors should be considered in making an intelligent selection of chocolate milk drinks. To answer this and other questions regarding chocolate milk, a study was undertaken in the Dairy Industry Department in cooperation with the Department of Bacteriology and Chemistry at the Massachusetts State College. In the experimental studies to date only commercial chocolate-flavored syrups and cocoa powders have been used as flavoring material for chocolate milk, but the use of chocolate liquor is contemplated.

Before discussing the experimental data, a few words should be said regarding the amount of chocolate milk consumed in the United States. To the author's knowledge there are no available statistics giving the yearly consumption of chocolate milk in the United States. However, a nation-wide survey on sales of chocolate milk for the year 1934, conducted by The Milk Dealer (1) gives some indication as to the relative amounts of chocolate milk consumed in various sections of the United States. This survey does not cover all distributors selling chocolate milk, nor does it include the chocolate milk which is made in the home, schools, etc. The amount of chocolate milk sold in various sections of the country, as reported in the survey, is as follows:

Section	*Number of dealers reported*	*Average annual sale of chocolate milk during 1934 (qts.)*
East	10	16,700
Middle West	21	42,252
South	7	15,500
Far West	14	68,827

Middle and far westerners appear to be more "chocolate milk conscious" than consumers in the remainder of the country.

The survey shows also that the sales outlets for chocolate milk over the entire country ranked in the following order of importance: Retail routes and stores tied for first place; schools, second; restaurants, third; factories, fourth; hotels and filling stations, tied for fifth; and roadside stands, sixth. It should be noted that the consumption of chocolate milk in schools is high.

EXPERIMENTAL

Flavor of Chocolate Milk

Flavor blend and intensity are usually among the chief considerations in selecting a chocolate milk. Since the ideal flavor varies with the individual taste of the consumer, not much can be done along experimental lines on this subject unless a large number of judges are available. When selecting a flavor, the guiding consideration should be not only its first appeal or flavor impression but also its probable effect on the amount consumed by steady chocolate milk drinkers. A highly flavored chocolate milk may appeal favorably at first but may become distasteful to steady users.

The Sanitary Quality of Chocolate Milk

This important factor is too often neglected when selecting a chocolate milk. The effect of the number and kind of micro-organisms in commercial chocolate syrups and cocoas on the sanitary quality of chocolate milk depends largely on the method used in making chocolate milk. Three general methods are in use: 1, adding a prepared syrup to pasteurized milk with no subsequent heating; 2, adding a prepared syrup to milk and then pasteurizing; and 3, adding the dry ingredients to the milk and heating to dissolve and to pasteurize the product.

There is less danger of increasing the bacteria count of chocolate milk from the chocolate syrup or cocoa powder if methods 2 or 3 are used. If the first method is used, considerable contamination from the chocolate syrup is possible. The first method is used to a large extent in localities where laws prohibit pasteurization of plain milk and chocolate milk in the same equipment. The small distributor usually finds that it is more economical to add prepared syrup to cold pasteurized milk than to buy additional equipment for processing chocolate milk. In order to find out just how much contamination might be expected a number of commercial chocolate syrups and cocoas were examined for bacterial content (by the Department of Bacteriology). The findings are reported in Table 1 which shows that considerable contamination may be expected from some of the chocolate syrups if the syrup is added after the milk has been pasteurized and cooled. For example, if the syrup containing 200,000 bacteria per gram is mixed with milk in the ratio of 1 to 10, then we may expect a bacterial increase of approximately 18,000 per gram of milk, which is significant. The first sixteen samples listed in Table 1 are chocolate-flavored syrups and the last eight are cocoa powders. Thirteen additional cocoa powders have been examined in the dairy laboratory and the plate counts ranged from 3,000 to 55,000 bacteria per gram.

Some of the high bacterial counts obtained in the cocoa powders may be due to the fact that some of the samples were not received in their original containers. However, this does not explain the high bacteria counts obtained in some of the syrups, as these samples were received in sealed containers. Directions for making chocolate syrups usually specify a heat treatment of 185 to 200 degrees F. for 15 to 30 minutes. This treatment should make the product sterile from a practical viewpoint. The author is not familiar with the heat treatment that is given to some of the prepared syrups on the market. However, no coliform bacteria or organisms producing hemolysis on blood agar were found, which indicates the absence of pathogenic organisms in the syrup and cocoas examined.

TABLE 1

The number and types of micro-organisms found in various commercial chocolate flavored syrups and cocoas and the pH of these products

Sample No.	Plate count	Yeasts and molds	Acid formers	Proteolytic forms*	Spore formers*	Coliform	Blood agar	Anaerobes	pH
16	5,000	20	80	Neg.	10	Neg.	Neg.	Neg.	......
52	1,800	10	30	Neg.	10	Neg.	Neg.	Neg.	...
53	35,000	400	700	10,000	10,100	Neg.	Neg.	30	6.5
63	200,000	10	100	10	100	Neg.	Neg.	...	5.3
54	800	80	20	100	100	Neg.	Neg.	10	5.9
64	200	20	40	100	100	Neg.	Neg.	.....	5.7
50	1,000	.....	.....	.....	.....	....	....	.....	.
55	900	100	10	10	10	Neg.	Neg.	10	6.5
51	1,600	40	20	100	100	Neg.	Neg.	Neg.	5.9
21	3,400	10	Neg.	500	100	Neg.	Neg.	20	5.8
56	20,000	Neg.	3,500	100	1,000	Neg.	Neg.	.....	5.2
57	50	Neg.	Neg.	10	10	Neg.	Neg.	Neg.	5.1
58	40	Neg.	Neg.	10	10	Neg.	Neg.	Neg.	5,8
59	20	Neg.	Neg.	10	10	Neg.	Neg.	Neg.	5.1
60	40	Neg.	Neg.	Neg	10	Neg.	Neg.	Neg.	6.2
62	1,100	10	20	100	100	Neg.	Neg.	.....	5.6
1	2,900	300	200	10,000	10,000	Neg.	Neg.	....	5.5
2	Millions	50	100	1,000	1,000	Neg.	Neg.	......	5.4
5	Millions	1,000	2,000	500	500	Neg.	Neg.	....	6.6
6	2,400	200	400	500	500	Neg.	Neg.	....	5.2
7	1,800	40	100	500	500	Neg.	Neg.	.....	5.6
8	1,600	800	100	1,000	1,000	Neg.	Neg.	......	5.6
19	25,000	1,000	4,000	1,000	1,000	Neg.	Neg.	.....	5.5
27	8,000	500	200	1,000	1,000	Neg.	Neg.	.....	7.1

Data obtained from R. L. France, Assistant Research Professor, Department of Bacteriology.

* The figures given are not counts but are the highest dilution in which these forms were found.

Table 1 also gives the pH of the various chocolate syrups and cocoas, which ranged from 6.5 to 5.1 for the syrups and from 7.1 to 5.2 for the powders.

Viscosity of Chocolate Milk

An excessive viscosity in chocolate milk is undesirable because it is generally associated with the following objectionable features in the product: 1, too rich appearance; 2, filmy coating after taste in the mouth; 3, appearance of gassy fermentation; and 4, wheyed-off appearance. Just how high the viscosity must be in order to be classed as excessive will again vary with the individual taste. In general the viscosity should be kept as low as possible without obtaining sedimentation.

TABLE 2

The effect of chocolate flavored syrups on some of the properties of milk

Chocolate flavored syrup[1] No.	Relative[2] viscosity of choc. milk	Cream layer	Sedimentation	pH of[3] chocolate syrup	pH of[4] chocolate milk	pH of[4] whey	Curd[5] tension	Relative[6] ease of mixing syrup and milk
	sec.	*cc.*	*cc.*					
1	40.4	0.0	Trace	5.91	6.50	5.93	0	++
2	26.5	1.0	2.0	5.85	6.49	5.95	20	++
3	26.4	1.5	0.0	6.34	6.59	6.08	0	+
4	26.4	1.5	0.0	6.51	6.62	6.08	0	+
5	26.8	2.5	1.0	5.70	6.48	5.90	19	++
6	29.7	1.0	2.0	5.82	6.45	5.95	15	++
Plain milk	24.0	1.5	0.0	.	6.53	5.91	40	...
7	27.5	2.5	1.5	5.19	6.34	5.89	25	++
8	26.2	1.5	2.0	5.09	6.47	5.98	23	+
9	27.3	1.5	2.0	5.75	6.47	6.00	18	+
10	26.1	2.0	1.5	5.14	6.43	5.98	23	+
11	25.9	2.0	1.0	6.23	6.57	5.91	26	+
12	45.4	0.0	0.0	5.60	6.50	5.92	8	++
Plain milk	24.4	1.5	0.0	. .	6.48	5.99	42	

[1] Used in the ratio of 1: 11 with milk–3.4 per cent butter fat.
[2] At 48° F. using pipette viscosimeter.
[3] Glass electrode.
[4] Quinhydrone electrode.
[5] Hill–Curd Meter.
[6] + indicates syrup mixes readily with cold milk.
++ indicates syrup mixes less readily with cold milk.

Table 2 shows that commercial chocolate-flavored syrups vary considerably in their ability to affect the viscosity of the chocolate milk. Some of the syrups almost doubled the viscosity of the milk while others increased it only very slightly. Table 3 shows that the suspending agent is chiefly

TABLE 3

The influence of the various components in chocolate milk on the curd tension and other properties of milk

Treatment	Relative[1] viscosity	pH before[2] adding acid	pH of[2] whey	Curd[3] tension
	sec.			
Whole milk— pasteurized[4]	23.6	6.70	6.20	19
Chocolate milk— “	51.0	6.85	6.25	0
Cocoa + milk— “	24.1	6.75	6.16	7
Sugar + milk— “	24.7	6.65	6.19	11
Sodium Alginate + milk— “	38.1	6.80	6.22	0
Water + milk— “	23.3	6.70	6.22	13
Whole milk—raw	23.4	6.70	6.23	37

[1] At 46° F. using pipette viscosimeter.
[2] Quinhydrone electrode.
[3] Used a pepsin-hydrochloric acid coagulant and the American Curd-O-Meter.
[4] 165° F. for 30 minutes.

responsible for the greater viscosity of chocolate milk as compared with plain milk.

Sedimentation in Chocolate Milk

Sedimentation in chocolate milk is undesirable because it detracts from the appearance of the product and also causes inconvenience in serving. The sediment is chiefly crude fiber which is insoluble in milk. Whether this crude fiber has any ill effects on the digestive system or on the digestibility of the milk is not definitely known. In some earlier experiments (2) with white rats which were fed milk containing 7 and 10 per cent of cocoa, it was noted that the indigestible cocoa fiber tended to block the intestinal tract. However, blocking of the intestinal tract was not observed when rats received chocolate milk containing ordinary amounts (one per cent) of cocoa.

Sedimentation may be prevented by the addition of a suspending agent or by using a chocolate syrup from which the crude fiber has been removed. A number of substances such as starches, gelatin and sodium alginate have been used as suspending agents. It is highly desirable to use a suspending agent which will prevent sedimentation without producing excessive viscosity in the product.

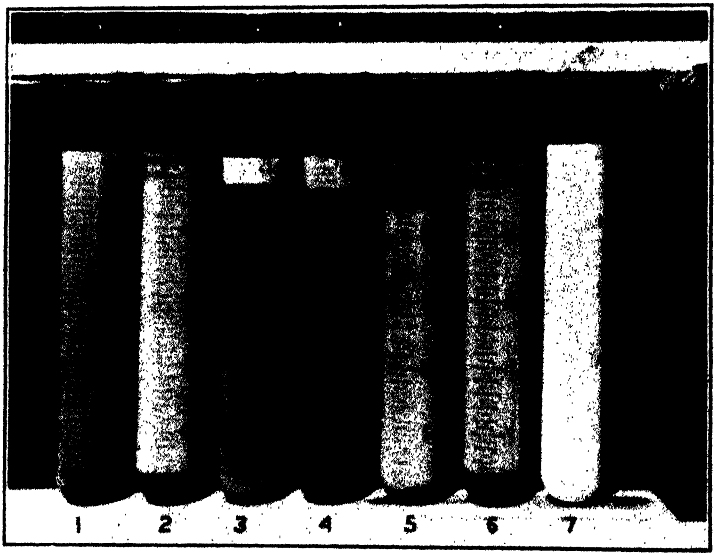

FIG. 1. Appearance of chocolate milk when using various commercial chocolate flavored syrups with a milk base containing 3.4 per cent butter fat.

The test tube at the extreme right contains plain milk, 3.4 per cent butter fat. The numbers on the other test tubes corresponds with the chocolate syrup numbers as given in Table 2.

Note the absence of cream layer and sediment in No. 1; the dark colored cream layer and the amount of sediment in No. 2, 5 and 6; and absence of sediment and the clear, creamy color of the cream layer in No. 3 and 4.

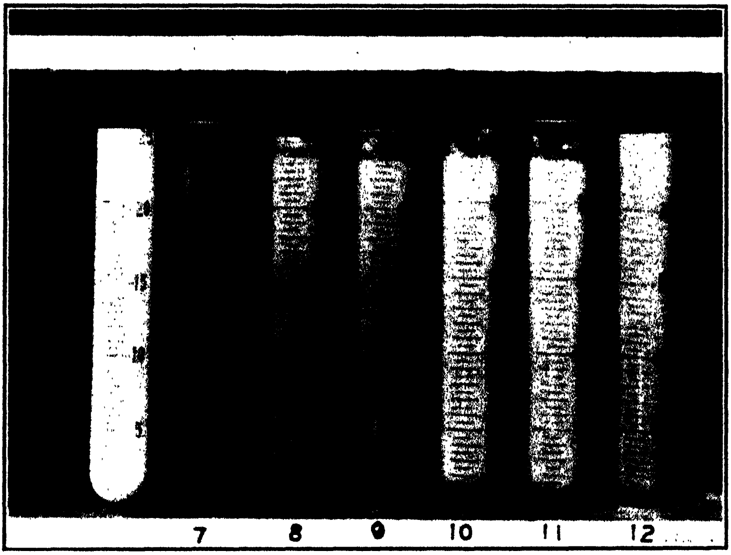

FIG. 2. Appearance of chocolate milk when using various commercial chocolate flavored syrups with a milk base containing 3.4 per cent butter fat.

The test tube at the extreme left contains plain milk, 3.4 per cent butter fat. The numbers on the other test tubes correspond with the chocolate syrup numbers as given in Table 2.

Note the dark colored cream layer and the sedimentation in No. 7, 8, 9, 10 and 11. No. 12 shows no cream layer or sediment.

Table 2 and Figures 1 and 2 show that 25 cc. portions of various chocolate milks in test tubes, after 24 hours, had sedimentations varying from no sediment up to two cubic centimeters.

The Cream Layer in Chocolate Milk

Cream layer or cream line when present may be either desirable or undesirable in chocolate milk, depending upon the kind of chocolate syrup used and also upon the individual preference. If the cocoa fiber has not been removed from the syrup, then a cream line is extremely undesirable because the cream layer will have an uneven dark or dirty-looking color which is sometimes mistaken for curd lumps. If a cocoa-fiber-free syrup is used, then the cream layer will have a more creamy color which has the advantage of indicating to the consumer the approximate amount of butter fat in the chocolate milk. The absence of a cream layer is preferred by many milk distributors.

The creaming of chocolate milk can be prevented by pasteurizing the milk at a high temperature or by homogenization. The addition of a suspending agent to prevent sedimentation also prevents creaming. Table 2 and Figures 1 and 2 show that 25 cc. portions of various chocolate milks in test tubes, after 24 hours, had a cream layer varying from no layer up to 2.5 cc.

The Reaction or pH of Chocolate Milk

Although the pH of the various chocolate-flavored syrups as given in Table 2 varied from 5.0 to 6.5, yet the pH of the chocolate milk remained nearly constant, varying only from 6.3 to 6.6. This is to be expected since milk is highly buffered.

Ease of Mixing Chocolate-Flavored Syrup With Milk

Although none of the chocolate-flavored syrups listed in Table 2 mixed with sufficient difficulty to make them objectionable, yet some differences were noted in this respect. Fine distinctions in mixing ability were not attempted. It is desirable when mixing syrup and milk cold to use a chocolate syrup which mixes easily. The inconvenience in mixing when using a syrup which does not mix readily often results in a product which is not uniform from day to day.

The Curd Tension of Chocolate Milk

The great amount of interest which the public has shown during the past few years in soft curd milk prompted the author to investigate the curd tension of chocolate milk. Though the consensus of opinion seems to be that soft curd milk is more readily digested by infants and people with impaired digestion than hard curd milk, it is questionable whether the curd tension of milk is a matter of practical importance for individuals on a mixed diet.

Table 2 shows that all of the chocolate syrups lowered the curd tension of plain milk, which had an original curd tension of 40 and 42 grams. Some syrups lowered the curd tension reading to 0, others only to 25 grams.

Table 3 shows the effect of the various components in chocolate milk on the curd tension, including the effect of a high pasteurization temperature. The lower curd tension of chocolate milk is not entirely due to the dilution of the milk with syrup, because when the milk was diluted with water the curd tension was found to be 13 grams as compared to zero grams for chocolate milk. The suspending agent sodium alginate was more effective in lowering the curd tension than either sugar or cocoa. The varying effect of the components in chocolate milk on curd tension cannot be explained by differences in pH. The results to date show no correlation between the curd tension and digestibility of chocolate milk as measured by animal experimentation and in vitro.

Effect of the Acidity of Milk on Chocolate Milk

Some distributors use an inferior grade of milk in their chocolate milk on the assumption that the chocolate or cocoa will cover the defective flavor of the milk. Table 4 and Figure 3 show what happens when milk of varying degrees of acidity is used with a specific chocolate syrup for making

TABLE 4

The effect of the acidity of skim milk, 2% b.f. milk, and 4% b.f. milk on the appearance and viscosity of chocolate milk[1]

Sample No.	Milk used	Acidity of milk	Relative viscosity[2]	Amount of separation[3]		
				After 1 day	After 2 days	After 4 days
		%	*sec.*	*cc.*	*cc.*	*cc.*
1	Skim	.16	31.9	2.5	5.5	9.0
2	"	.18	31.6	4.0	8.0	11.5
3	"	.20	31.3	7.0	9.5	13.0
4	"	.24	30.0	11.0	14.0	15.5
5	2% b.f.	.16	37.2	0.0	0.0	0.5
6	"	.18	35.5	0.0	Trace[4]	2.5
7	"	.20	33.7	0.0	4.5	6.0
8	"	.24	31.9	5.5	8.0	10.5
9	4% b.f.	.16	46.3	0.0	0.0	0.0
10	"	.18	42.3	0.0	0.0	Trace[4]
11	"	.20	38.9	0.0	0.0	Trace[4]
12	"	.24	36.4	0.0	7.0[4]	10.0

[1] Used a commercially prepared chocolate flavored syrup, 1 to 11.

[2] Determinations made with pipette viscosimeter at 52° F. after samples had aged for 24 hours at 38° F.

[3] Lightest colored portion.

[4] Separation not distinct.

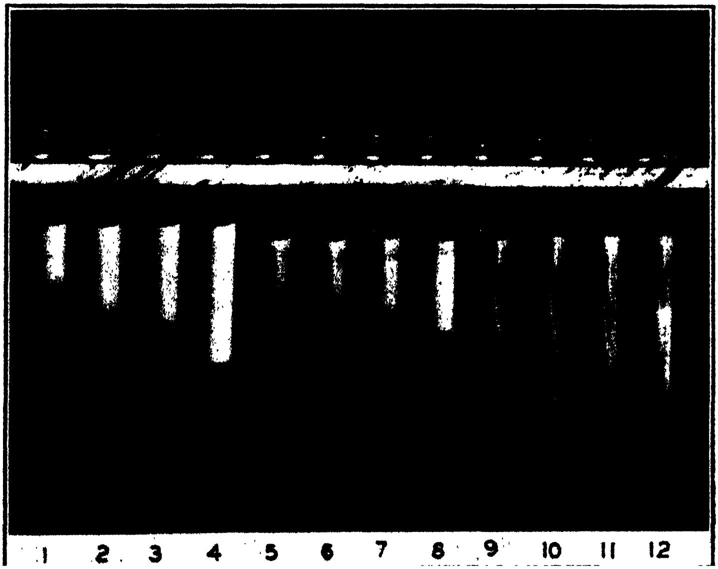

FIG. 3. The effect of the acidity of skim milk, 2 per cent butter fat milk, and 4 per cent butter fat milk on the appearance of chocolate milk.*

Acidity	Skim milk Test tube no.	2% butter fat milk Test tube no.	4% butter fat milk Test tube no.
.16%	1	5	9
.18%	2	6	10
.20%	3	7	11
.24%	4	8	12

* The white portion shows the amount of separation of chocolate syrup from the milk.

chocolate milk. Increasing the acidity of milk from .16 per cent to .24 per cent decreased the viscosity of the chocolate milk. This decrease became more marked as the butterfat content of the milk was raised. An increase in acidity was also found to increase the separation in chocolate milk and to a greater degree as the percentage of fat was decreased in the milk.

Effect of Cocoa on the Nutritive Value of Milk as Determined by Animal Feeding

A comparative study of the total nutritive value of chocolate milk and plain milk has been made when both milks were supplemented with iron, copper, manganese, and sugar, and fed as an exclusive diet to white rats. When the animals received equal quantities of plain milk and sugar but varying amounts of cocoa, the one per cent cocoa diet was equal to the plain milk diet; the two and one-half per cent cocoa diet gave questionable retardation in rate of growth, and the four per cent cocoa diet definitely retarded growth. However, in a later experiment it was found that when cod liver oil and brewers yeast were added to the milk diets, the four per cent cocoa diet was equal to the plain milk diet. These results are given in Table 5.

TABLE 5

Effect of cod liver oil and yeast as supplements to a chocolate milk diet

Diet	Gain after 6 weeks—grams	
	Without cod liver oil and yeast	With cod liver oil and yeast
No cocoa control	60.5	64.5
1 per cent Dutch process cocoa	60.0	....
2.5 per cent Dutch process cocoa	55.2	....
4 per cent Dutch process cocoa	47.2	65.3
4 per cent American process cocoa	....	60.5

In order to secure information on the physiological action of cocoa, urine analyses were made. Samples over a period of 24 hours were collected from 12 animals, four rats on each of the following diets: No cocoa, four per cent Dutch process cocoa, and four per cent American process cocoa. The average results for five trials are given in Table 6. The increase in volume of urine for the chocolate milk diets is probably due to the theobromine and caffeine which are known to have a diuretic action. The cocoa diets also

TABLE 6

Effect of chocolate milk diets on the composition of urine

Kind of cocoa	Volume per day	Specific gravity	Acidity .1 N NaOH	pH	Uric acid mg. per
	cc.		*cc.*		cc.
No cocoa (control)	4.6	1.043	2.3	6.72	0.2064
4 per cent Dutch process cocoa	5.7	1.037	2.6	6.49	0.1913
4 per cent American process cocoa	5.6	1.034	2.4	6.45	0.2023

produced a urine which was somewhat higher in acidity. No significant differences were noted in the uric acid content of the urine.

Fecal pH determinations using the quinhydrone electrode on samples collected from rats receiving no cocoa, four per cent Dutch process, and four per cent American process cocoa, showed no marked differences.

Effect of Cocoa on the Digestibility of Milk in Vitro

The purpose of this study was to check, if possible, the results on the animal feeding experiments. Digestion experiments have been made with milk and chocolate milk containing one and four per cent cocoa. Digestion was carried out with artificial gastric juice, in a rotating machine set in an incubator at 37 degrees C., in order that digestion might go on under conditions somewhat approaching those of the stomach. Samples were removed before digestion and after digesting for two, four, and six hours, and were then analyzed for non-protein nitrogen. Preliminary results are given in Table 7. The data show that milk alone gives the greatest total increase in non-protein nitrogen, the four per cent chocolate milk the least, and the one per cent chocolate milk only slightly less than the plain milk. In general these results check with the results obtained by animal feeding experimentation.

TABLE 7*

Effect of cocoa on the digestibility of milk in vitro

Kind of milk	Non-protein nitrogen per 100 cc. of milk			
	Initial	After 2 hrs.	After 4 hrs.	After 6 hrs.
	mg.	*mg.*	*mg.*	*mg.*
Plain milk	23.2	266	311	332
Plain milk + 1 per cent cocoa	22.8	248	302	309
Plain milk	24.6	277	329	351
Plain milk + 4 per cent cocoa	23.8	244	280	294

* Data obtained from Leo Lipman, graduate student in Department of Dairy Industry.

Effect of the Addition of Vitamins to Chocolate Milk

Chocolate-flavored syrups and cocoa powders supplemented with various vitamins have been introduced commercially. A commercial chocolate-flavored syrup containing yeast has been assayed for both vitamins B and G, and was found to contain not less than ten international units of B and not less than eleven Bourquin-Sherman units of G per fluid ounce.

Figure 4 shows the comparative growth of rats fed plain milk (4 per cent butterfat) and chocolate milk (one part chocolate-flavored syrup containing yeast and eleven parts of milk—4 per cent butterfat). The total nutritive value of each kind of milk was measured by exclusive feeding of the milk supplemented with minerals to twelve albino rats. The rats were fed in accordance with the principle of paired feeding. Both groups received the same amount of milk daily, since the milk intakes were equalized within groups. From Figure 4, it is evident that the animals receiving the chocolate milk made considerably greater gains in weight than animals on the plain milk diet. The chocolate-flavored syrup used in this experiment has been reported to contain 3.25 calories per gram. On the basis that four per cent butterfat milk contains 314 calories per pound, 100 grams of plain milk would contain approximately 70 calories as compared to approximately 90 calories per 100 grams of chocolate milk. The growth curves reflect these differences in the caloric value of chocolate milk and plain milk.

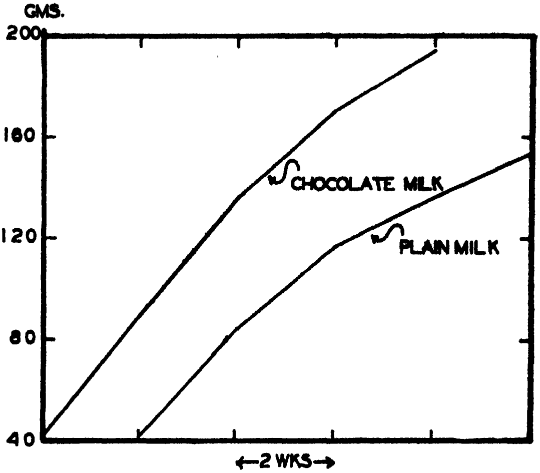

FIG. 4. Comparative growth of rats fed equal amounts of plain milk (pasteurized whole milk—4 per cent butter fat) and chocolate milk (one part chocolate flavored syrup containing yeast plus 11 parts of pasteurized whole milk—4 per cent butter fat). Each curve is the average for 12 animals.

SUMMARY

A good chocolate milk should have the following properties:

1. A flavor which appeals to the majority of steady consumers.
2. A low bacteria count. (The maximum bacteria count should comply

with state and municipal laws for the grade of plain milk which is used as a base in the chocolate milk.)

3. No excessive viscosity. (Viscosity should be kept as low as possible without obtaining sedimentation.)

4. Sedimentation is considered undesirable.

5. Cream layer may or may not be present. If cream layer is present, it should be cream colored and not darkly colored or mottled.

6. To avoid excessive sweetness, preferably not over 5 per cent of sugar should be added.

7. The milk used in chocolate milk should not be over .2 per cent in acidity and preferably lower.

8.* A total fat content of at least equivalent to the butterfat required by the State standards for plain whole milk. Total fat then includes the butterfat normally found in the whole milk base and the cocoa fat normally found in the chocolate flavoring material.

9. Cocoa should be used only in such quantities that the digestibility of the milk is not lowered. Approximately 1 per cent by weight of cocoa, in the finished product, does not adversely affect digestibility and is sufficient for flavoring the product.

CONCLUSIONS

(The investigation is being continued and obviously some of the following conclusions must be considered only as preliminary.)

1. Animal feeding experiments indicate that ordinary amounts of cocoa (approximately 1 per cent) do not decrease the nutritive value of milk.

2. Digestion experiments in vitro indicate that ordinary amounts of cocoa (approximately 1 per cent) do decrease the digestibility of the milk slightly, but probably not enough to be of practical significance.

3. Animal feeding experiments, and in vitro digestion trials indicate that four per cent of cocoa in milk definitely decreases the digestibility of the milk.

4. All of the chocolate-flavored syrups and cocoas studied so far have decreased the curd tension of milk. No direct correlation, however, has been found between the curd tension and nutritive value of chocolate milk, as measured by animal feeding experiments and in vitro digestion trials.

5. The pH of chocolate-flavored syrup and cocoa powder had very little effect upon the pH of the chocolate milk.

6. The sanitary quality of some commercial chocolate-flavored syrups examined was poor. If they were to be added to cold milk they would seriously increase the bacterial content of the chocolate milk.

7. Bacteria counts were high in some of the cocoa powders examined, yet

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* Refers to the product made from a whole milk base, and does not include "chocolate-flavored dairy drinks."

this is not as serious as for chocolate-flavored syrups, because the cocoa is heated in the preparation of the syrup and less powder is used than syrup.

8. No pathogenic organisms were found in any of the chocolate-flavored syrups or cocoas.

9. Bacteriological standards for chocolate milk equal to those already required for plain milk would be desirable.

10. Standardizing the composition of chocolate milk would be desirable because it would make the product more uniform and would protect the consumer. However, more experimental data are necessary before such standards can be formulated.

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GRASS SILAGE: A CRITICAL REVIEW OF THE LITERATURE*

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The problems of preserving roughages for the winter feeding of livestock are perennial. The preservation of legumes and grasses as hay of high quality is governed mainly by two factors, namely, ideal curing weather, and stage of maturity of the crops. The inability of the livestock man to control these factors results in heavy nutrient losses in the hays during the curing process due to leaching and the loss (shattering) of leaves.

The development of an alternate process of preservation has been the objective of investigators for years. Artificial dehydration of the hay crops shows some promise but, as yet, the process involves a large capital outlay and high operating costs. This at present eliminates it from consideration by the average livestock man.

Preserving these crops as silage seems to be the most logical approach to the solution of this problem. If they can be preserved as silage and stored irrespective of weather, the common haymaking losses will be reduced to a marked extent. However, early attempts to ensile crops of high protein content such as legumes usually met with failure. In many instances an unpalatable silage resulted with a putrid odor and an excessive loss of nutrients. These undesirable factors were attributed by Aldershaw (2) and Whittet (109) to a low content of fermentable carbohydrates in the ensiled material coupled with the stage of growth and the moisture content of the plant material. In trying to improve the quality of the ensiled material, six methods of preservation have been investigated. The principles involved were:

1. The addition of various acids to the green material as it was being ensiled which thereby increased the acidity of the mass to a point where undesirable bacterial action was checked (22).
2. The addition of fermentable carbohydrates to the fodder which favored the desirable lactic acid fermentation and resulted in a natural increase in acidity (82, 110).
3. The inoculation of the fodder with a culture of lactic acid-forming bacteria to increase the number of desirable bacteria which caused a more rapid fermentation and thereby checked the activity of the undesirable organisms (47).
4. The partial wilting of the grasses before ensiling to reduce the bacterial activity (113).

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5. The replacement of the entrapped air with carbon dioxide immediately after ensiling to eliminate plant cell respiration and aerobic bacterial activity (42, 87).

6. The killing of the bacterial flora by sterilization (107).

The first recorded attempt to preserve fodder by the addition of acid was made in 1885 by Giglioli (22), when beet leaves were ensiled by the addition of hydrochloric acid. His product showed approximately 2.2 per cent acidity calculated as lactic acid and in which there was a loss of 11.8 per cent of the digestible nutrients. Little or no additional work was done on the acid method until 1925 when Virtanen began his classic work. His work led to the A.I.V. method (7, 74, 77, 89, 98, 99, 101, 102, 103) which was first put to a practical test in 1928 (102) on two farms in Finland.

The A.I.V. method consists of the addition of a mixture of equal quantities of sulfuric and hydrochloric acids in a 2 normal solution to the fodder as it is ensiled. Sufficient acid is added to bring the acidity of the mass between pH 3.6 and pH 4.0. Virtanen (104) postulated the use of sulphuryl chloride (SO_2Cl_2) which undergoes hydrolysis and yields sulfuric and hydrochloric acids.

This process was followed by the use of a mixture of hydrochloric and phosphoric acids and "Penthesta" solution. The "Penthesta" solution is a solution of phosphorus pentachloride in water which by hydrolysis yields hydrochloric and phosphoric acids (24, 84, 85, 86).

At present, work is being carried on in which phosphoric acid is the sole preservative. Phosphoric acid, or mixtures containing phosphoric acid, not only have a preservative effect on the silage, but also act as sources of phosphorus for animal nutrition and enrichment of the manure (78).

In 1917, Reed and Fitch (82) observed, "When a legume like cowpeas is siloed alone a poor quality of silage is made, but when cowpeas are siloed with some other crop, such as corn, a first-class silage is produced." This observation led to the idea that a silage of good quality might be made from legumes by the addition of a source of readily fermentable carbohdyrate. Reed and Fitch (82) in ensiling trials with alfalfa found "that the addition of materials such as molasses, corn chop and other supplements containing a high percentage of carbohydrate material was effective in preserving the alfalfa as silage." Swanson and Tague (90), working at the same time, found molasses to be the best of the carbohydrate preservatives. Hunter (60) found that the addition of carbohydrates to alfalfa produced a silage with a higher acid content and with a reduced breakdown of the protein as compared with the same type of silage which contained no added preservative.

Following these observations the next attempt in the use of sugar as a preservative was the Defu (29) method. This method not only utilizes sugar but also hydrochloric acid and sometimes phosphoric acid. The acids are used to increase the acidity to pH 4 and the sugar supplies a substrate so that the lactic acid bacteria, through the formation of lactic acid, are able to increase the acidity to approximately pH 3.5 (29, 43, 45, 50, 70, 80, 81, 107).

The use of dairy by-products, such as whey, has been suggested (5, 6, 27, 28). The lactose present in the whey serves as an excellent substrate for the lactic acid bacteria but due to the large quantity required or to the necessary partial drying so as not to add too much water to the fodder, whey is impractical for common use.

The use of molasses as a source of fermentable carbohydrate seems at present to be the most desirable method which involves the addition of carbohydrates (6, 13, 15, 18, 32, 41, 43, 55, 60, 110). The molasses most generally used is a by-product of sugar refineries. It contains approximately 65 per cent soluble carbohydrates of which from 55 to 60 per cent is fermentable sucrose. Because of its high sugar content, it may be used at full strength or diluted to suit the user. Molasses, like other sources of carbohydrates, serves not only as a preservative, but also as a cheap source of additional nutrients.

Inoculating silage with organisms which produce lactic acid has been suggested (47). The method proposes the addition of a pure culture of a lactic-acid-former which causes a more rapid acid fermentation and thus checks the action of undesirable organisms. Watson (107) stated, "The addition of cultures of lactic organisms has never been accompanied by tangible improvements since the crop usually has a sufficiently large natural lactic-acid-organism flora of its own."

Woodward and Shepherd (113) stated, "It is apparent that the exclusion of air is the only condition required to prevent molding or rotting of silage." They further stated that, "The extent and character of the fermentation, however, is profoundly affected by the moisture content of the material. The higher the moisture content, the more extensive the fermentation and the greater the likelihood that objectionable odors will develop." They advocate a partial wilting of the grass prior to ensiling to a dry matter content of at least 30 per cent. Their results showed that "Partial drying of legumes before chopping for the silo increased the surface spoilage, increased the temperature of fermentation as much as 9° F., and improved the palatability of the silage."

Mikhin, Fokin and Tupikova (73) reported that in grass silage with a normal moisture content carbon dioxide had no inhibiting effect on bacterial activity but if the moisture content was low the gas had a strong inhibitory effect. They also found that green silage with a moisture content of 50 to 55 per cent developed only one-half to one-third the acidity of normal silage.

Bender *et al.* (18) in an ensiling study with a high-moisture grass (60 to 75 per cent moisture) and a low-moisture grass (23 to 55 per cent moisture) found that the low-moisture material developed a temperature of 160° F. and showed considerable charring while the high-moisture material showed a maximum temperature of 109° F. and no charring. They further found (14) that the high temperatures developed with low-moisture material could be reduced if sufficient pressure was developed on the mass to exclude the air. However, the wilting of the green material prior to ensiling reduced the carotene content to a marked degree (91).

Erf (42) is of the opinion that if fodder is packed in air-tight containers at a low temperature and with an atmosphere of non-oxidizing gases a palatable, well-preserved product results. He found that packing the material in air-tight receivers or silos with dry ice was a desirable method of preserving legumes. He reports that this silage is very palatable and that it is a good preservative of the "fertility factor." The fertility factor referred to is the alkaloid, betain. The replacement of the entrapped air with carbon dioxide in the ensiled mass was attempted on both a large and a small scale by Schmidt (87). The method was one in which the air in the filled silo was evacuated and the space refilled with carbon dioxide. Although the fodder seemed to keep well under laboratory conditions, there was a distinct formation of butyric acid in the large silo which was thought to be caused by an incomplete evacuation of the mass. The value of creating an anaerobic condition lies in the fact that plant cell respiration and other aerobic activities such as those of the yeast and molds will be eliminated. Such a condition, however, will be favorable for the growth of desirable or undesirable types of anaerobic organisms. Unless conditions are favorable for a rapid formation of lactic acid only a poor quality silage can possibly result due to the action of putrefactive types of bacteria.

The use of antiseptics was first suggested in 1886 (107) when carbon bisulfide was used to check respiration. Since then attempts have been made to sterilize the fodder by heat produced by steaming the silage in the silo and also through the use of electrical current. Chemical sterilizing agents such as formaldehyde, formic acid, and Torosilon, which liberates a sterilizing gas, have been used but the results did not warrant their further use. "Complete sterilization of the mass can be dismissed from the mind as a practical alternative in making silage," according to Watson (107).

The process of ensiling in its essentials involves a storing of the fodder with the expulsion of air through pressures developed by the mass of material. This is followed by a series of reactions involving normal plant cell respiration which utilizes oxygen and in turn is succeeded by reactions caused by anaerobic bacteria. The type of bacterial action depends to a large extent on the type of material ensiled. The desirable type of action is that which results in the rapid formation of lactic and acetic acids from the fermentation of carbohydrates. When an acidity of pH 3.5 to 4.0 is reached the action of putrefactive bacteria and bacteria giving rise to butyric acid is checked (104).

The course of reactions in the ensiling process may be classified under two general headings; aerobic and anaerobic. When fodder is placed in the silo there is a certain amount of air entrapped. Until the oxygen of this air is consumed, the plant cell-respiration continues (104) and yields mainly carbon dioxide and water; aerobic organisms such as the yeasts and molds continue to multiply. When the conditions become anaerobic, the aerobic organisms, particularly the yeasts and molds, although considered dead and unable to reproduce, still function as enzyme systems and continue to carry on transformations, such as the formation of alcohol and other metabolic end-products (106). It is, therefore, essential to exclude as much air as possible when filling the silo so as to limit the number of yeasts and molds present by limiting the time during which they are able to multiply (59). Some of the metabolic products of aerobic activity such as ethyl alcohol may be oxidized to acetic acid under the ensuing anaerobic conditions.

When the available oxygen of the air has been completely utilized, the anaerobic organisms commence their fermentative reactions. The most important of the anaerobic organisms are the acid-forming (59) and the proteolytic bacteria (4, 102). The proteolytic organisms are able to transform proteins to such products as ammonia, amino acids, amines, and amides. As these are metabolic waste products, they will be formed in greatest quantity when the nitrogen requirements of the organisms are lowest. They are most apt to occur when the organism is using protein as an energy source as well as for the synthesis of cell substance. Two conditions will prevent to a great degree excessive protein decomposition. First, the addition of utilizable carbohydrates suppresses the utilization of protein for energy. In this case protein will be utilized only for the building of cell material for the bacteria. In this form the protein is still available as nutrient material for the animal. Second, controlling conditions by adjusting the acidity of the silage through the addition of acids will inactivate proteolytic bacteria and their enzymes. However, the addition of fermentable carbohydrates checks the decomposition of protein in both ways in that it supplies a source of energy for the bacteria and adjusts the acidity of the silage by the bacterial formation of lactic and acetic acids. Silage with an adequate carbohydrate supply shows a predominance of lactic-acid-formers (5).

The optimum pH for silage preservation is between 3.5 and 4.0 (104, 105). When the pH of the mass is 3.5 the rate of the plant cell respiration is reduced to 10 per cent of the rate at a pH approximating neutrality (104). The harmful bacterial action which gives rise to the formation of butyric acid or the breakdown of protein to undesirable products is completely inhibited at a pH of or below 4 (104). As it has been shown that the spores of butyric-acid-formers can tolerate an acidity of pH 3.5 (96) it is necessary to maintain an acidity at a pH of or below 4 so that these spores do not regerminate into active organisms.

In all processes of ensiling green fodder there are losses of dry matter. These losses may be partially accounted for by such volatile materials as carbon dioxide, hydrogen, methane, and ammonia which are the result of bacterial action and of plant cell respiration. Drainage from the silo carries with it a portion of the soluble material. The addition of preservatives is not only an attempt to make a more palatable feed but also to reduce these common losses to a minimum with special emphasis on the loss of volatile materials. It will be seen, therefore, that in order to minimize these losses the fodder must be stored compactly so as to exclude as much air as possible. Cell respiration is thereby reduced. The mass of silage must also be acidified to stop undesirable bacterial activity. The use of preservatives at present has the sole objective of increasing the acidity of the mass.

Watson (107) stated, "The loss in the ordinary process of silage varies from 25 to 35 per cent of starch equivalent, according to the container used and general quality of the final product. The loss of digestible crude protein may be as high as 40 per cent, particularly in stacks or clamps, but may be as low as 10 per cent in some of the best silages made in a suitable container."

Camburn, Ellenberger, Newlander, and Jones (31) in a comparison of four different methods of preserving grasses found that, "The nutrient losses from alfalfa and timothy due to sun curing (A), artificial drying (B), ensiling without molasses (C), and ensiling with molasses (D), only those occurring in the body of the silo being considered, and excluding top spoilage, on the percentage basis were:

	A	*B*	*C*	*D*
Dry matter	15.8	5.0	9.3	8.2
Ash	21.5	9.9	1.1	6.8
Crude protein	22.8	16.7	5.8	8.0
Crude fiber	11.8	+1.0	1.9	0.6
Nitrogen-free extract	13.8	3.0	17.9	13.9
Ether extract	28.4	4.5	+21.8	+15.6"

They stated that, "These data indicate that the molasses nutrients were not wasted during the ensiling process and that only enough were fermented to produce a desirable amount of acidity." A comparison of ordinary silage (no preservative added), A.I.V. silage, and molasses silage with regard to the nutrient losses by Davies, Botham and Thompson (35) showed the following results:

	**Ordinary*	*A.I.V.*	*Molasses*
Dry matter (crude)	24.1	18.1	12.5
Ether extract (crude)	+53.2	+28.6	+35.3
Fiber (crude)	24.0	23.0	5.8
Protein (crude)	28.2	11.4	8.2

* Figures are percentage losses on the dry basis unless otherwise stated.

A 110 per cent gain in digestible ether extract was noted in the ordinary silage, and a 63.6 per cent gain in A.I.V. silage acidified to pH 3.5. Ordinary silage had a 22.6 per cent reduction in digestible fiber, A.I.V. silage 17.1 per cent, and molasses silage 0.6 per cent. While the A.I.V. silage showed the lowest loss of crude and digestible protein in comparison with molasses silage (11.4 per cent and 7.8 per cent, respectively), the molasses silage showed the smallest loss of dry matter (12.5 per cent).

Davies, Botham, and Thompson (35) found that the order of digestibility based on dry-matter content places the ordinary silage lowest (60.3 per cent), the A.I.V. highest (71.8 per cent), and the molasses intermediate (64.3 per cent). According to Watson and Ferguson (108) ensiling increases to some extent the digestibility of the crude fiber and crude protein when mineral acids, molasses, or whey are used as preservatives.

Bechdel (10) in 1923 found that "roughage composed of clover silage and hay was decidedly better for milk production than hay alone as the sole roughage." There is, however, no significant increase in the butter fat content of the milk (21, 58).

The feeding of silage has been shown not to increase the acidity of milk over that of cows on a silage-free diet (71).

Butter produced from the cream of cows which were fed silage was harder and had a better consistency than that from either pasture-fed cows or from cows which received dry feed (19, 56). According to some workers (12, 72, 84) milk produced by silage-fed cows could not be used in the making of hard or fatty cheeses. If the silage which was fed had undergone a butyric acid fermentation the milk produced was not suitable for the making of soft cheeses, and resulted in a "split" or over-fermented product. The use of inorganic acids as fodder preservatives seemed more likely to produce a butyric fermentation in cheese than did the use of organic preservatives (97).

Garrett, Hartman, and Arnold (46) reported that molasses grass silage is superior to either corn silage or beet pulp in the quality of milk produced with regard to initial flavor, stability of flavor in storage, and stability toward the catalytic effects of added copper on the formation of an "oxidized" flavor. This stabilizing effect is thought to be due, at least in part, to the higher carotene content of the grass silage. The color intensity of the milk was found to be greatest when grass silage was fed, intermediate when corn silage was used as the roughage, and lowest when the cows were receiving beet pulp. A correlation was found between color and stability of the milk in resisting the development of off-flavors. Hartman (52) found that milk produced by cows fed second-cutting alfalfa silage preserved with 100 pounds of molasses per ton had a color value only 4 per cent lower than milk produced on fresh early pasture.

Of the vitamins, three have been found to be present in various silages. These were A, B_2, and C (100). As it is supposed that cattle are able to synthesize B_2 and C, the important vitamin supplied by the silage is A. Taylor (91) has shown that the carotene of silage acted as vitamin A in feeding trials with white rats. The determination of carotene in silages, therefore, appears to be a reliable index of its vitamin A potency. The carotene content of grass silage was found to be twice that of artificially-dried alfalfa hay which had been stored for the same period of time (91). It has been reported that the carotene content of silage is higher than for the corresponding green material (69, 79). However, it is still doubtful as to whether this is an actual increase in carotene or an increase in some other substance which is determined along with the carotene in the procedure used. There is the possibility that the carotene is more readily extracted from the preserved material than from the fresh grasses. Wiseman, Kane, Shinn, and Cary (111) have found that other pigments in the carotene extract of corn silages and hays, which are determined as carotene in the usual method of analysis, amounted to from 11 to 32 per cent of the total extracted pigments.

The vitamin A potency of the milk of cows fed A.I.V. silage equaled in some cases that of the milk of cows on pasture. In all cases the silage produced a milk of higher vitamin A content than did field cured hay (54, 79, 100). Both A.I.V. and molasses silage were found to be superior to silage with no preservative as a source of vitamin A (53).

Brouwer (26) found that the feeding of silage preserved with the use of mineral acids caused an increase in the acidity and ammonia content of the urine. Silages of high acid content (pH 2.2) preserved with mineral acids, fed in moderate amounts, increased the acidity and ammonia content to a greater extent and caused acidosis and an increase in the chloride content of the blood. These results are avoided by feeding sodium bicarbonate in sufficient quantity to neutralize the mineral acids in the fodder. Calcium carbonate frequently is not as effective in producing the desired results.

Crosemann (33) reported that when silages preserved with the addition of hydrochloric and sulfuric acids were fed to animals there was an increase in the ammonia and calcium content and a decrease in the bicarbonate content of the urine and the urine became acid. When large amounts of chloride ions were ingested, as in silage preserved with hydrochloric acid, there was a diminution of the alkali reserves of the blood plasma and an increase in the acidity. These conditions were not produced when silages with sulfuric acid as the preservative were fed.

When grass silage preserved without mineral acids was fed, the pH and carbon dioxide content of the urine were lowered slightly below that of animals fed hay but the pH of the urine of the silage-fed animals remained alkaline. From this it may be concluded that the organic acids which result from carbohydrate fermentation in silage are, for the most part, oxidized in the animal body and do not give an undesirable effect in the urine and blood plasma (30).

Bohstedt, Rupel, King, Peterson and Hegsted (20) found that, "As to any noteworthy physiological effect of A.I.V.-alfalfa silage on the cows, the results of the first experiment are of interest. The alkaline reserve of the blood was lowered on an average 10 cc. per cent during the A.I.V. feeding period. The pH of the urine dropped from 7.5 to 5.9, and the ratio of ammonia nitrogen to the total nitrogen was increased from 0.6 per cent to 11.0 per cent, indicating that the cows used ammonia to aid in neutralizing the acids present. These changes are more or less insignificant, and normal relationships were established within two weeks after the feeding of the A.I.V.-alfalfa silage was discontinued."

When silage is fed in moderate amounts there is no influence on the utilization of calcium, phosphorus, and nitrogen nor is the calcium: phosphorus ratio affected. If, however, silage contains 2 or more per cent of hydrochloric acid there may be an adverse affect. The increased urinary calcium may affect the calcium: phosphorus ratio. However, this may be corrected by the feeding of calcium phosphate, calcium carbonate, or hay or fresh fodder rich in minerals. The addition of calcium phosphate, although improving the calcium and phosphorus balance, decreased the digestibility and utilization of protein (48, 49, 62, 64, 65, 66, 88).

Dairy cattle and calves fed clover silage in which there had been a conversion of 34.4 per cent of the protein to water-soluble compounds such as amides showed a utilization of the amide nitrogen (67). Hart, Bohstedt, Deobald, and Wegner (51) have shown an increase in weight in calves when urea or ammonium carbonate is added to a diet which contains only sufficient protein to maintain the weight of the animal. They conclude, "These results will bear directly upon certain problems now before our farmers. First, in the making of molasses legume silage some of the protein nitrogen is converted to ammonia. It is apparent that this degradation may not mean a complete or even a partial loss of nitrogen. Second, it is also apparent that fertilization of pastures with nitrogen salts, whereby the nitrogen in the plant is appreciably increased, would mean that this increased nitrogen could in all probability be utilized by this type of animal just as well as if it were finished protein. Third, it is entirely possible that the improvement of a low protein ration of poor biological value can be made through the use of molasses and urea; that the proteins formed in these bacterial cells may supplement the rest of the ration better than some protein concentrates that are now in use. This is a problem for the future."

Crops which have grown coarse and rank and as a result would make an inferior hay may be made into silage which can be fed with much less waste (63). Brouwer (25) is of the opinion that it is preferable to make hay of grasses when the weather is satisfactory but if poor haying weather prevails, the making of silage is preferred. When grass is to be used as silage it is preferable to cut the grass at an earlier stage of growth than for hay.

The highest yield of protein per acre is obtained, for most crops, at the first-heads-out stage (36). As the plant grows past this stage, the crude protein decreases and the crude fiber and nitrogen-free extract contents increase.

Alfalfa-molasses silage seems to be superior to alfalfa hay for milk production when the intake is calculated on the dry-weight basis (57). A feeding trial by Horwood and Wells (58) has shown that cows which were fed silage consumed more digestible protein and less total digestible nutrients than did cows fed hay, yet they produced more milk although there was a greater weight gain with the hay-fed group. In experiments with calves, it was found that 25 pounds of grass silage had practically the same feeding value as 10 pounds of good hay from the same meadow (37).

The list of crops* adaptable to the making of grass silage includes cultivated and non-cultivated ones and both annuals and perennials. In general it may be said that all crops which can be made into hay can be stored as grass silage. This would include legumes, grasses, and cereals. The cereal crops such as oats, wheat, barley and rye when harvested in the green or dough stage of growth are very satisfactory. A palatable silage of excellent quality may be made of any combination of green crops that may be desired. The primary desire is to obtain the highest possible yield of nutrients, especially protein, coupled with a low cost of production.

The legumes, especially alfalfa with its high protein content, high yields, and desirable fertilizing effect on the soil, are usually the more preferable. The cereals, which are annuals are less desirable except where they are used as nurse crops for new seedings of alfalfa, clovers and timothy. The most preferred annuals are soybeans or mixtures of soybeans and Sudan grass, peas and oats, and wheat and vetch which give large yields of high quality fodder.

In conclusion it should be noted that many of the questions in connection with the preservation of crops as silage remain unanswered. The field of experimentation in this connection is a fertile one from the standpoint of both pure and applied research as can be seen from this review.

There can be no doubt as to the practical value of ensiling hay crops, either, 1—to provide hay insurance, 2—to use as summer feed when pastures are short, or 3—to replace corn silage in the diet of cattle where soil erosion is a serious factor in farm operations. The one fact which should be kept in mind continually is that ensiling methods developed or to be developed must be simple in operation and positive in action under farm conditions.

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* See reference numbers 1, 3, 8, 9, 11, 16, 17, 23, 34, 38, 39, 40, 44, 61, 68, 75, 76, 83, 92, 93, 94, 95, 111, 114.

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THE THIRTY-FOURTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ
Secretary-Treasurer

The American Dairy Science Association was called to order by the President, Earl Weaver, in the Administration Auditorium at the University of Idaho, on Tuesday, June 27, at 1:30 P. M. for the thirty-fourth annual meeting.

The program printed in the June issue of the JOURNAL OF DAIRY SCIENCE was prepared by the Program Committee. The June issue also contains the abstracts of the papers presented.

Dean E. J. Iddings, College of Agriculture, University of Idaho, and President E. O. Holland, State College of Washington, were each separately introduced. They officially welcomed the Association membership to their respective campuses.

President Earl Weaver gave the following response:

PRESIDENT'S ADDRESS

"The cordial official welcome we have just heard, together with the fine hospitality we have already encountered since our arrival at Pullman and Moscow convince all of us that this thirty-fourth annual meeting of the American Dairy Science Association will constitute a memorable occasion for each of us individually and for the Association. I am sure I have the enthusiastic endorsement of all the members here when I express to you of Idaho and Washington our appreciation for the efforts you have expended to make our visit a pleasant and profitable one. It should be remembered also that some of our members have found it impossible to be here on this occasion. Many of them communicated to me their regrets that forbidding circumstances arose. I take this occasion to express their best wishes to you of the local committee and to the other members who are here.

The American Dairy Science Association this year has concluded a third of a century of those services for which it was founded. At various times in the Association history new projects and objectives have been inaugurated. Some of these projects have been of a transitory nature and have been entirely accomplished within a longer or shorter period. Others of them have been of a permanent and continuous nature. They demand constant attention. As president of the Association I wish to report that the activities of your officers during the current year have been largely directed to the advancement of these permanent, major objectives of the Association. I do not report any new projects inaugurated during the year.

The Journal: A report on the JOURNAL OF DAIRY SCIENCE will be presented next Friday by Mr. Sutton, the editor. I am convinced the membership will find great satisfaction in this report. However, there are three matters pertaining to the Journal to which I wish to call your attention. The editor, the secretary of the Association and the Journal Management Committee have rendered valiant service. Mr. Sutton in his first year as editor has shown the enthusiasm, patience and diligence so necessary in that position and we are all reassured as to the welfare of the Journal, which must be acknowledged as the major project of the Association.

Then there is a second Journal matter which I feel has not been sufficiently emphasized to our members. Our technical workers recognize the suitability of the Journal as a medium for the publication of their results. Apparently, however, many of these workers fail to recognize the feasibility and economy in ordering liberal numbers of "reprints" for subsequent use in handling requests. Department heads and station directors should be advised of the economies possible through this procedure. The greater use of Journal reprints could save materially in printing costs to the station.

The third matter pertains to advertising. While few of us are familiar with the varied phases of advertising and in many cases are not very sympathetic, we must all realize that the advertising accounts make possible the continuance of the Journal. Our successes in selling space to those concerns more directly related to Dairy Manufactures have been gratifying. To Mr. Stoltz is due the credit for these favorable responses that have been enjoyed. However, the Journal Management Committee is faced with the problem of selling space to concerns identified with Dairy Production. Certain ones of our members, who are in a position to render service in this direction, must be called upon to help solve this problem.

Membership: The number of members in the Association at the present time is the highest we have ever had. This fine record is due to the membership chairmen in the several states. Of course, an especial concern in a membership campaign is to get new members. This year several of the states obtained in excess of 20 new members each. Ohio reported 45 and Pennsylvania took the lead with 74. The state chairman are all deserving of our praise.

A total of 387 new members was obtained. On the nomination blank which the new member submits he indicates his particular field of interest. A preponderance of the new members expressed interest in Dairy Manufactures. They out-numbered those interested in Dairy Production by nearly a 2 to 1 ratio. Every member of the Association who is able to exert some influence should encourage his friends in Dairy Production to identify themselves with the Association. There is a particular opportunity to secure members from among those qualified persons who are identified with larger farms and breeding establishments, with commercial feed companies and companies handling grinders, silo equipment and other equipment. This field for members has not yet been effectively canvassed. It offers us an opportunity.

Student Affiliates: During past years a few of our college workers have

DR. E. S. GUTHRIE, PRESIDENT ELECT

actively encouraged their students while yet in college to affiliate themselves with the Association. Some of us have been only moderately active in such a program. There are several reasons why we should encourage student affiliation. A student in college finds satisfaction in the fact he is identified with his professional society. Furthermore, upon graduation, there is an appeal for him to continue his status as an active member. A good number of student affiliates each year is a quite effective safe-guard for the Association in subsequent years. I believe we have testimony for this in our records. Those states this year which have been most successful in reporting new members are those states wherein active Junior Chapters are operating in the college or university.

A year ago President Gregory appointed a committee of J. A. Nelson, E. V. Ellington and A. A. Borland to formulate uniform rules whereby Junior Chapters could be sponsored. This committee report will be submitted for your action at the general meeting on Friday.

The Dairy Curriculum: A year ago a comprehensive committee was appointed to study the Dairy Curricula in the different institutions and if found feasible to formulate proposals that might be embraced in our constant efforts to make our courses of study fit the needs. The committee has encountered extreme diversity of opinion, which is not at all unexpected. They have a prolonged task before them but I am confident their deliberations and proposals will prove helpful to all who have responsibility in constructing our courses.

On Friday, Dr. Roadhouse of the sub-committee on the Dairy Manufactures Curriculum will present a progress report. A companion report from Professor Wylie for the sub-committee on Dairy Production will also be presented.

Regulations for the Borden Awards: During the year a committee consisting of Harold Macy, F. B. Morrison and H. A. Ruehe has assembled opinions from every Dairy Department and from individuals pertaining to the rules to be observed in the Borden Awards. This committee has its proposed rules ready for submission at the next general session on Friday; the action of the Association on these will be expected.

Members' proposals of policy changes: I have been impressed this year with the extreme interest on the part of several members of the Association as evidenced by proposals they have submitted to change some of the Association policies and invoke new ones. Those proposals always receive attention from your officers and directors. I should like to encourage all the members to submit their ideas and opinions for the betterment of the Association.

Recognition of Personnel: In attempting to handle the duties of the president's office this year I have been more forcefully impressed than ever before with some of the problems inherent in our rapidly expanding organization. Particular reference is made to the fact that our membership is not acquainted sufficiently with their own organization and its many committees, sections and divisions.

While the situation is not unexpected we are attempting to combat it by presenting to the membership at this meeting the committeemen and the officers of the sections and divisions. We feel the service these men render is deserving of our official recognition. Furthermore, it seems entirely appropriate to make this gesture for a little wider acquaintanceship between all the members and all the officers of our Association.

In line with the same objectives we are also recognizing today the services rendered by former presidents of our Association. They have all retained their interest in the Association; many continue to render conspicuous official service. To them is due the credit for the foundation upon which our Association has been built.

Then I want to take this occasion to acknowledge the fine services the present directors and committee members have performed this year. They have been most willing to assume some tedious duties assigned them; they have accomplished their tasks most effectively. I am sure too I express to these officers the gratitude of the entire membership for the services they have rendered."

Past President H. W. Gregory was then called upon to introduce the past presidents as follows:

1907–1908	R. A. Pearson	1926	J. B. Fitch
*1909–1910	C. H. Eckles	1927–1928	G. C. White
1911–1912	O. F. Hunziker	1929	J. M. Sherman
1913–1914	J. H. Frandsen	1930	H. B. Ellenberger
*1915–1916	Fred Rasmussen	1931	E. L. Anthony
*1916–1917	Wm. A. Stocking	1932	H. C. Jackson
1918	A. C. Anderson	1933	R. B. Stoltz
1919	M. Mortensen	1934	C. L. Roadhouse
*1920–1921	C. H. Eckles	1935	H. A. Ruehe
1922–1923	A. A. Borland	1936	R. R. Graves
1924–1925	O. E. Reed	1937	H. W. Gregory

Mr. Gregory read greetings from the following Past Presidents: A. C. Anderson, M. Mortensen, J. B. Fitch, G. C. White, J. M. Sherman, H. B. Ellenberger, E. L. Anthony, H. C. Jackson, H. A. Ruehe and R. R. Graves.

He then introduced the following Past Presidents: O. F. Hunziker, J. H. Frandsen, A. A. Borland, O. E. Reed, R. B. Stoltz and C. L. Roadhouse.

Vice President E. S. Guthrie introduced the officers of the Sections and Divisions:

H. W. Cave, Chairman—A. H. Kuhlman, Vice Chairman of the Production Section.

P. A. Downs, Chairman—F. H. Herzer, Vice Chairman of the Manufacturing Section.

S. J. Brownell, Chairman—O. J. Hill, Secretary of the Extension Section.

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* Deceased.

Richard E. Waters, Secretary of the Southern Division.

I. H. Loughary, Chairman—G. A. Richardson, Vice Chairman and J. O. Tretsven, Secretary of the Western Division.

President Weaver then introduced the Directors who were present:—H. W. Gregory, E. S. Guthrie, R. B. Stoltz, J. W. Linn, E. V. Ellington, Harold Macy and E. G. Hood.

President Weaver announced the following committees:

Resolutions Committee—C. Y. Cannon, D. R. Theophilus, B. E. Horrall, L. S. Palmer and Otto Hill.

Nominating Committee—H. C. Jackson, P. S. Lucas, O. E. Reed, I. R. Jones and C. A. Smith.

Committee on Borden Nominations—Prescribed by rules to consist of current chairman and two immediate past chairman of respective sections.

Manufactures:

P. H. Tracy—Chairman, Mfg. Section 1936–37.
C. J. Babcock— " " " 1937–38.
P. A. Downs-- " " " 1938–39.

Production:

F. W. Atkeson—Chairman, Prod. Section 1936–37.
W. E. Krauss— " " " 1937–38.
H. W. Cave— " " " 1938–39.

Committee on Borden Awards—(Each president appoints one member to serve three years.)

Production:

H. B. Ellenberger—term expires Dec. 31, 1939; appointed by Graves.
F. B. Morrison—term expires Dec. 31, 1940; appointed by Gregory.
L. W. Morley--term expires Dec. 31, 1941; appointed by Weaver.

Manufactures:

H. A. Ruehe—term expires Dec. 31, 1939; appointed by Graves.
M. Mortensen—term expires Dec. 31, 1940; appointed by Gregory.
W. D. Dotterer—term expires Dec. 31, 1941; appointed by Weaver.

H. E. Lattig, Assistant Dean of Agriculture, University of Idaho, was introduced and read a paper entitled "Objectives of Dairy Husbandary Teaching in Secondary Schools."

E. V. Ellington, Vice Dean and Director of the College of Agriculture and Experiment Station, State College of Washington, presented a paper "Adult Education in Dairy Husbandry."

There were one hundred and sixty-nine in attendance.

GENERAL BUSINESS MEETING

AMERICAN DAIRY SCIENCE ASSOCIATION

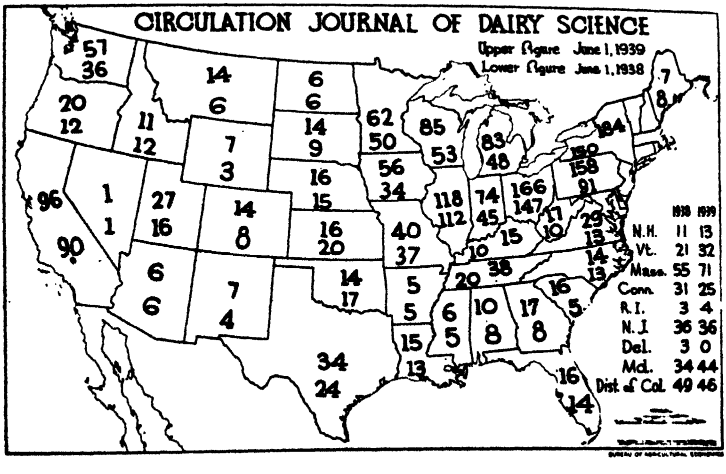
PULLMAN, WASHINGTON, JUNE 30, 1939

President Earl Weaver called the meeting to order at 9:30 A.M. in Science Hall on the State College of Washington campus.

The secretary-treasurer read the financial report which had previously been approved by the Board of Directors. Upon motion, duly seconded, the report was accepted and referred to the auditing committee.

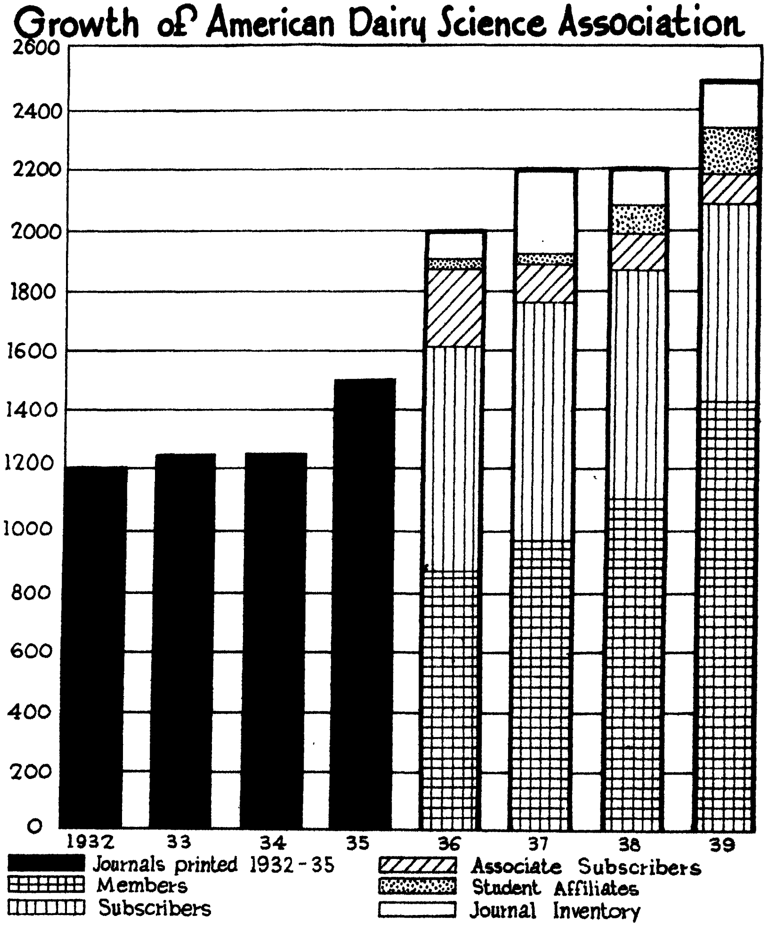
> "February 20, 1939
>
> To the Members of the American Dairy Science Association
> Gentlemen:
>
> Mr. Walter C. Burnham of Columbus, Ohio, certified public accountant, has made an audit and report of the financial condition of the Association.
>
> The Auditing Committee has conferred with Mr. Burnham and is satisfied that he has made a careful examination of all the assets and liabilities of the Association and that all the accounts are accurate. The committee is satisfied that the balance sheet and related summary of profit and loss fairly represents the financial condition of the American Dairy Science Association.
>
> Respectfully submitted,
> J. F. Lyman (Signed)
> S. M. Salisbury (Signed)
> L. H. Burgwald (Signed)
> *Auditing Committee*
> *American Dairy Science Association"*

Circulation of Journal: The secretary then called the members' attention to a chart showing the growth of circulation during the past six years. He also showed a map of the United States giving the circulation of the Journal



by states on June 1, 1938, and June 1, 1939. These figures accompany this report.

"The results of the chairman of the state campaign committees are very gratifying. May I congratulate the members who have been instrumental in bringing about the large increase of members during the past year.



Under our new constitution we are especially anxious to interest the output of the dairy schools in student affiliates. We have a total of 149 student affiliates this year and are of the opinion that we should have not less than 500. Pennsylvania has 40, Ohio 34, Tennessee and Michigan 11 each, Indiana 9, Texas 8, Vermont 6, Utah and Massachusetts 5 each. Many of our larger dairy schools do not have any student affiliates. This can be done by the faculty selling their student body on the need of their possessing their Journals of Dairy Science the same as their text books.

The success and progress of the dairy industry will depend largely upon the viewpoint that the graduates of the dairy schools of today have towards research. Research is not only for full-time men paid for that job alone, but it is for every technically trained man both in the field of dairy production and dairy manufacturing. Up to this age most research has been made on mechanical things, but the next quarter century it is going to be more complicated, requiring training in chemistry, physics, bacteriology and other technical sciences. This will require college-trained men. Do not you in the field of education feel responsible for your graduates after they leave you and enter the industry?

In order to qualify for the needs of the present and future they should be sold on the need of reading the JOURNAL OF DAIRY SCIENCE.

Advertising: Our income from advertising has been on the increase, but it is not near as much as it should be. In 1936 we had 80½ pages, in 1937, 108 pages, in 1938, 94 pages and for the first six months of 1939, 61 pages. We should be advertising equipment and supplies for the field of production. The cattle clubs and breed organizations have not seen fit to advertise in our Journal."

The Secretary then read the minutes of the Board of Directors meetings as follows:

MEETING OF BOARD OF DIRECTORS

AMERICAN DAIRY SCIENCE ASSOCIATION

8:00 P.M., June 26, 1939

A meeting of the Board of Directors of the American Dairy Science Association was held in Troy Hall, Monday, June 26, 1939, at 8:00 P.M.

Present: President, Earl Weaver; Vice-President, E. S. Guthrie; Secretary-Treasurer, R. B. Stoltz; Directors, E. G. Hood, E. V. Ellington, H. W. Gregory, Harold Macy, J. W. Linn.

Absent: Directors, C. E. Wylie, M. E. Parker.

O. F. Hunziker, Chairman of the Journal Management Committee, submitted the following report:

"Your Committee on Journal Management beg to respectfully submit the following brief report:

Journal Finances: The details of Journal Finances for the year 1938 are contained in the very complete, audited, annual report submitted to your Board by the Secretary-Treasurer, January 31, 1939.

It is significant to note here that each one of the three major sources of revenue of the Association, i.e., membership fees, subscriptions and advertisements, showed a very substantial increase over the previous year. The total circulation of the Journal for 1938 passed the 2000 mark, being 2053. The financial figures for 1938 speak for themselves. The gratifying situation which they reveal is no accident.

As to the year 1939, the prospects look indeed encouraging. The Secretary-Treasurer's office reports a total Journal circulation for this year up to June 5th of the record-breaking figure of 2285.

Editorial Department: A report of details of the editorial phase of the Journal, showing number of pages for manuscripts, abstracts, review articles, etc., distribution of articles relative to branches of industry and by States, together with numerous timely suggestions in the interest of increased service of the Journal to its readers, has been prepared by the Editor for presentation to the Board at one of the sessions of this meeting.

In the interest of economy of time the present report will, therefore, omit editorial statistics and will briefly refer to the Editor's suggestions under their respective subjects, in later paragraphs. Your committee desires to take this opportunity, however, to express our admiration and appreciation of the splendid manner in which our new editor has taken hold of his arduous task, and of his able performance of every phase of editorial duties. He has proved himself eminently qualified for the job, as tangibly demonstrated by the continued high quality of the Journal. It has been a pleasure to work with him, and he richly deserves our expression of recognition of his successful efforts.

Reprinting of Journal Articles in other Publications: Requests from other publishers for reprinting articles that have appeared in the JOURNAL OF DAIRY SCIENCE in their publications are not numerous, but whenever they occur they raise the question of Policy. It is the judgment of your committee that the occasional reproduction of Journal articles in another recognized publication, is in no way objectionable and tends to promote desirable publicity for our Journal. We feel, however, that there should be established a definite policy on this point, that has the full approval of this Board. We therefore, respectfully request that the Board of Directors take action on the following recommendations:

1. That requests for the reprinting of Journal Articles in other reputable publications be granted if printed in its entirety;
2. That the question of suitableness of the publication making the request be left to the discretion of the Secretary-Treasurer; and,
3. That the JOURNAL OF DAIRY SCIENCE insist that the author's name and the phrase 'by Permission of the JOURNAL OF DAIRY SCIENCE' appear in legible type directly under the title of the reprinted article, giving volume and inclusive paging.

Publication of Necrologies: The consideration of the publication of necrologies, and their lengths, is a problem that confronts the Journal recurrently. At times it becomes embarrassing to our editor. While it appears eminently fitting to make mention in the Journal of the passing of leaders in the dairy industry, and to do honor to the memory of members of our own Association, your committee feels that the JOURNAL OF DAIRY SCIENCE is not the proper organ for lengthy necrologies. In order to safeguard the proper purpose of the Journal and to avoid embarrassment, your committee respectfully request that this Board give consideration to the following recommendations:

1. That necrologies acceptable for publication in the Journal be limited to members only;

2. That necrologies not exceed 1500 words; and

3. That a Necrology Committee be appointed with instructions to provide the editor with appropriate necrologies of members who have passed on, and with suitable notices of the demise of other outstanding leaders of the dairy industry.

Standardizing Style of Manuscripts: There is undeniable room for improvement of the style of the articles that reach the editor. This refers to the physical make-up of articles, tables, etc. There is need especially also for greater uniformity of style of articles and tabular material. In order to work toward constructive improvement along this line there should be prepared for the Journal a brief style standard that may be placed at the disposition of authors of articles for the Journal. This matter is referred to in more detail in the Editor's report to the Board. The Editor, in fact, recommends that a committee be appointed with instructions to prepare a brief style standard, printed copies of which may be distributed among our membership.

Your committee on Journal Management is in full sympathy with this suggestion and we beg to respectfully recommend the appointment of such a committee. We further recommend that the completed draft of the brief style standard be submitted to the Editor and to the Journal Management Committee for their approval, and that separate prints of the approved standards be supplied to the entire membership.

Changing of Abstract Page Numbers: In order to eliminate possible confusion in page numbers, between articles and abstracts, all abstract page numbers are now prefixed by the letter "A." The change commenced with the January number of the Journal, so that in the XXII volume (1939) all abstract page numbers will be found prefixed with the letter "A."

Changing Color and Style of Lettering of Cover Page of Journal: Your committee recommend that the question and character of change of color and style of lettering the cover page of the Journal be left to the discretion of the Journal Management Committee.

Respectfully submitted by

A. A. Borland,
R. B. Stoltz,
O. F. Hunziker, *Chairman*
The Committee on Journal Management.

Upon motion duly seconded, the report was accepted.

Mr. Harold Macy, Chairman of the Committee on Borden Awards, then submitted the following report and upon motion duly seconded the report was adopted as amended, with the understanding that it was to be printed in the Journal and reprints made for distribution:

REGULATIONS GOVERNING THE BORDEN AWARDS FOR RESEARCH IN

(*a*) DAIRY PRODUCTION
(*b*) DAIRY MANUFACTURING

FOREWORD

The Borden Company in 1936 made the following offer to the American Dairy Science Association:

"An annual award of $1,000 in cash and a gold medal is offered by the Borden Company for each of the purposes set forth herewith and administered as outlined:

(1) For work in the production field, breeding or feeding of dairy cattle, farm sanitation or quality production, etc., to be administered by the American Dairy Science Association.

(2) For work in the processing field, such as improvement in equipment or methods in the handling of milk or cream and the production of milk products, to be administered by the American Dairy Science Association."

REGULATIONS

Eligibility: To be eligible for a Borden Award, a nominee shall have made a meritorious scientific contribution, or discovery pertaining to dairy production or dairy manufacturing, or invented a plan, process or device which is useful, valuable or significant in the theory or practice of dairy production or dairy manufacturing. The work, upon which the award is to be based, must have been completed or published during the five year period immediately preceding January first of the year during which the award is to be made. Under special circumstances, consideration also may be given to related work accomplished by the nominee during a period not to exceed ten years previous to the time the award is to be made. In judging the significance of the contribution of the nominee special consideration will be given to the independence of thought and originality manifested in planning and carrying out the research, the comprehensive nature of the work and its value to the industry. Anyone is considered eligible who is a living citizen of the United States or Canada and who at the time of nomination is actively engaged in the line of research for which the award is made. There are to be no restrictions as to race, sex or age. The award may be made to co-workers when deemed advisable. Membership in the American Dairy Science Association is not a requisite of eligibility. No individual shall receive the award more than once.

Nominations: A nomination for the award may be sent to the Secretary of the Association by any member of the Association except members of the

Award Committee. Nominations must be accompanied by a brief biographical sketch of the nominee and a list of publications, with reprints if possible, or other evidence of the completion of studies, with specific reference to the research upon which the nomination is based. No member may offer more than one nomination in any given year. No solicitation on the part of any individual or institution on behalf of any nominee will be tolerated. All nominations must be in the hands of the Secretary on or before January first of the year of the award. The Secretary will transmit all nominations as received to the Chairman of the Nominating Committee of the Production or Manufacturing Section depending upon the field of study of the nominee.

Nominating Committee: There shall be two Nominating Committees of three members each, one Committee representing the Production Section and the other the Manufacturing Section, in each case consisting of the Chairman of the Section for the current year and the last two preceding Chairmen of the Section. The senior member of each Committee shall serve as Chairman of the Committee. Any vacancy on such Committees may be filled by appointment by the President of the Association. All committee members must be members of the Association.

It shall be the sole duty of each Nominating Committee to receive the respective nominations for its section from the Secretary and to canvas the field when necessary in order to see that no one who has accomplished outstanding research has been overlooked and to nominate any worthy person or persons not nominated by the members of the Association. The Nominating Committee shall ignore all unsolicited support for any nominee beyond the original letter of nomination from an individual member.

Award Committee: There shall be two Award Committees of three members each, one Committee representing the Production Section and the other the Manufacturing Section. One member of each Committee shall be appointed by the President each year for a three-year period. The senior member of each Committee shall act as Chairman of the Committee. No member of the Committee shall serve more than one term. Any vacancy on such Committees may be filled by appointment by the President of the Association. All Committee members shall be members of the Association. If the name of a member of an Award Committee is included among the nominees of the Nominating Committee, such member shall withdraw from the Award Committee for the current year and the President of the Association shall appoint a member to fill the vacancy and to serve for the current year only. Each Award Committee shall act as the sole and final judge in selecting the recipient of the award from a list of nominees submitted by the Nominating Committee of the Section involved. The Award Committee should seek further evidence from the industry as to the effect which the work of any nominee may have had upon the actual practices in the industry. If an Award Committee shall decide that no outstanding work has been presented for its consideration, the award shall not be made. The Award Committee shall report their selection of recipients to the Secretary on or before June first of the year in which the award is to be made.

No two members from the same institution or business organization shall serve on any one Nominating or Award Committee. The President of the Association shall appoint a properly qualified member to fill any vacancy caused by the operation of this provision.

The announcements and the Awards shall be made at the Annual Meeting of the Association.

Respectfully submitted,
H. A. Ruehe
F. B. Morrison
H. Macy, *Chairman*

The Board was then adjourned.

MEETING OF BOARD OF DIRECTORS

AMERICAN DAIRY SCIENCE ASSOCIATION

10: 00 A.M., June 27, 1939

A meeting of the Board of Directors of the American Dairy Science Association was held in Troy Hall, Tuesday, June 27, 1939, at 10: 00 A.M.

Present: President, Earl Weaver; Vice President, E. S. Guthrie; Secretary-Treasurer, R. B. Stoltz; Directors, E. G. Hood, E. V. Ellington, H. W. Gregory, Harold Macy, J. W. Linn.

Absent: Directors, C. E. Wylie, M. E. Parker.

Mr. John Nelson, Chairman of the Committee on Junior Chapters of the American Dairy Science Association, then gave the report of his committee, a copy of which was distributed to each member in attendance.

Upon motion duly seconded the report was accepted.

President Weaver invited the Association to meet at Michigan State College in 1942. Letters were read from Mr. Weaver, Dean Ernest L. Anthony and President R. S. Shaw confirming the invitation to meet at East Lansing, Michigan, in 1941 or 1942. It was moved and seconded that the invitation from Michigan be placed on file and a letter expressing our appreciation be sent to the Dean and President. It was thought that the Board had no right to set the place of meeting this far in advance.

It was moved and duly seconded that a letter from Professor Borland of Pennsylvania State College regarding the change of time of holding the annual meeting be answered by the secretary and that the secretary be authorized to make a survey among the various schools in regard to changing the time of holding our annual meeting.

The Board of Directors then adjourned.

MEETING OF BOARD OF DIRECTORS
AMERICAN DAIRY SCIENCE ASSOCIATION

3:30 P.M., June 27, 1939
Pullman, Washington

A meeting of the Board of Directors of the American Dairy Science Association was held in Troy Hall, Tuesday, June 27, 1939, at 3:30 P.M.

Present: President, Earl Weaver; Vice President, E. S. Guthrie; Secretary-Treasurer, R. B. Stoltz; Directors, E. G. Hood, E. V. Ellington, H. W. Gregory, Harold Macy, J. W. Linn.

Absent: Directors, C. E. Wylie, M. E. Parker.

Mr. O. E. Reed then appeared before the Directors and invited the Association to meet in Washington and at the University of Maryland in 1941.

Letters were read from Mr. O. E. Reed of the U. S. Department of Agriculture, Bureau of Dairy Industry, Kenneth C. Ikeler, Head of the Animal and Dairy Industry Section and the Chamber of Commerce, Washington, D. C., confirming this invitation.

Mr. O. M. Camburn of the University of Vermont appeared before the Directors and invited the Association to meet in Burlington, Vermont, in 1941. A letter was read signed by H. B. Ellenberger of the University of Vermont giving information as to the facilities they had to offer.

Mr. J. H. Frandsen of Massachusetts State College then appeared before the Board and invited the Association to meet at Amherst, Massachusetts in 1941.

Upon motion duly seconded, the invitation from Vermont was accepted.

The meeting was then adjourned.

MEETING OF BOARD OF DIRECTORS AMERICAN DAIRY SCIENCE ASSOCIATION

9:00 A.M., June 29, 1939

The fourth meeting of the Board of Directors of the American Dairy Science Association was held in Troy Hall, Thursday, June 29, 1939 at 9:00 A.M.

Present: President, Earl Weaver; Vice-President, E. S. Guthrie; Secretary-Treasurer, R. B. Stoltz; Directors, E. G. Hood, E. V. Ellington, Harold Macy, J. W. Linn.

Absent: H. W. Gregory, C. E. Wylie, M. E. Parker.

The minutes of the three previous board meetings were read and approved.

Mr. Ellington then presented the following report for the Program Committee:

To the Board of Directors of the American Dairy Science Association

The Program Committee for the 34th Annual meeting of the American Dairy Science Association consisted of P. A. Downs, University of Nebraska; H. W. Cave, Kansas State College; S. J. Brownell, Cornell University and H. P. Davis, University of Nebraska; with H. P. Davis, University of Nebraska and T. S. Sutton, Ohio State University as Advisory Members and E. V. Ellington, Chairman. The first three members were members by virtue of their position as chairmen of the Manufacturing, Production and Extension Sections respectively. The personnel of this committee is selected in accordance with the recommendations of the Program Committee of 1937. The Chairman was appointed by the President of the Association on the recommendations of the Dairy Departments of the two host institutions.

The Chairman again was at a disadvantage in that the other members of the Committee were located far distant from the place of meeting. The Chairman had considerable preliminary correspondence with the other members, the concensus of opinion being that under the circumstances the local chairman would have to use considerable discretion in the preparation of the program. In order to divide this responsibility the Chairman selected three local committees on program consisting of the Production, Manufacturing and Extension members of the University of Idaho and the State College of Washington staffs.

The first call for titles of papers was made January 13, in a letter addressed to Heads of Dairy or Animal Husbandry Departments, in which the suggestion was made that each head call a staff meeting to discuss the proposed papers that might be presented from his institution. Attention was also called to them that the February number of the JOURNAL OF DAIRY SCIENCE would make the first call for papers, abstracts of which should be in the hands of the Program Chairman by April 15. Letters were also sent to the Bureau of Dairy Industry, United States Department of Agriculture.

On March 8, 1939, all members of the Association were contacted and again on March 23, the heads of departments were reminded that abstracts to be presented be in the hands of the Program Chairman by April 15.

Papers were not all received until April 17 and very few before April 14.

As a result a total of 101 papers were received, distributed as follows:

General 2, Manufacturing 37, Production 41 and Extension 21. These, with two exceptions, were included in the Association program.

The Committee commends the Extension Section on their method of organizing very definitely the type of program, and as a section, they presented to the Chairman of the Program Committee the subjects to be presented and the personnel to present them.

The Committee recommends that the Departments of Dairy Husbandry or Divisions from the various Experiment Stations exercise the same care in reviewing subject matter of the individuals presenting papers as they would if such papers were to be presented in the most conservative scientific journals. This would relieve the Committee of considerable responsibility in attempting to pass on suitability of material to be given.

Upon motion duly seconded, the report was adopted and the Board expressed their thanks and gratitude for the splendid work that the committee had rendered.

It was moved and seconded that the Board express their appreciation to the local people and that the copy of the resolutions regarding the appreciation be sent to the deans and presidents of the two institutions.

The meeting was then adjourned.

EDITOR'S REPORT

1. *Summary of Journal contents.*

During the past year, July, 1938 to May, 1939 inclusive, the JOURNAL OF DAIRY SCIENCE has carried 91 papers exclusive of the proceedings of the annual meeting, announcement material, necrologies, membership and circulation lists and abstracts. These 91 papers occupied 800 pages.

A classification of these papers shows that 52 papers covering 482 pages were devoted to manufacturing, 28 papers covering 212 pages to production and 8 papers occupying 66 pages were of basic interest to both divisions. In addition, two review articles have been published occupying 26 pages, also a history of the Students' National Contest in Judging Dairy Products covering 14 pages.

During the same period 126 pages have been devoted to announcement material, necrologies, membership lists, index, etc., and 232 pages were occupied by abstracts of literature. This is 1158 pages in all. This does not include the June, 1939 issue which contains the program and abstracts of papers.

2. *Distribution of papers on subject matter basis.*

We realize that it is difficult to classify many papers on the basis of subject matter. Some papers are of interest and contain information of value in several divisions of the industry. The classification presented here places the paper in the group where, in our judgment, it held the greatest interest.

SUBJECT MATTER DISTRIBUTION OF PAPERS

Subject Matter	*No. of Papers*
Milk	23
Chemistry and Analytical Methods	10
Butter	9
Cheese	8
Feeding, including effect on milk	8
Breeding	5
Physiology and Nutrition	5
Fluid Cream	4
Milk Secretion	4
Ice Cream	3
Condensed and Dry Milk	3

Analysis of Feeds	2
Bacteriology	2
Herd Management	2
Dairy Cattle Disease	1
Miscellaneous	2
	91

3. Contribution by states.

We thought it might be of interest, and perhaps stimulating as well, to see where the papers carried by the Journal originated. We appreciate that this sort of data, collected over a one year period, does not accurately measure the activity of a research institution. It may so happen that quite a number of investigations are completed in one year at a given institution and in the year following only a few investigations may yield data complete enough to justify publication. Again, certain institutions may not use the JOURNAL OF DAIRY SCIENCE so regularly as a place in which to publish their work, as others do, feeling perhaps that the nature of their work makes it of greater interest to other publications.

During the past year (exclusive of the June, 1939, issue) twenty-four states, the U.S.D.A. and Canada have contributed to the Journal. The number of their contributions is shown in the accompanying table:—

CONTRIBUTION BY STATES

State	*No. Papers*	*State*	*No. Papers*
New York	15	California	2
Illinois	10	Idaho	2
U.S.D.A.	7	Maryland	2
Wisconsin	7	New Jersey	2
Pennsylvania	6	Oklahoma	2
Iowa	4	West Virginia	2
Kansas	4	Canada	1
Michigan	4	Florida	1
Arizona	3	Montana	1
Indiana	3	Nebraska	1
Minnesota	3	Nevada	1
Missouri	3	Oregon	1
Ohio	3	Virginia	1

4. Review articles.

It was the original intention of those who formulated the plans for carrying review articles in the Journal that about six reviews should be published each year. We have been unable to meet with this schedule since only two review articles have been submitted to date, both of which have been published. The reviews that have been published have been so well received that we consider the project worthy of continuation. The publication of up to six reviews a year will be provided for should they be received. We recognize that the preparation of a comprehensive review worthy of that name is a laborious and time consuming task, and to many is an added responsibility to an already heavily loaded schedule.

5. *A 20 Year Index.*

The Journal Management Committee is considering plans for indexing the first 20 volumes of the Journal. Believing that such an index, if properly prepared, would be a valuable contribution to Dairy Science literature we recommend that the Board of Directors consider the plan presented by the Journal Management Committee and take action as their judgment directs.

6. *Suggestions for improvement.*

During the past year a number of embarrassing errors have occurred in the Journal. While we are not attempting to dodge our responsibility, we do believe that some of these errors would have been avoided had the authors carefully checked their manuscript before submitting it. This is particularly true in the case of reference citations. Some errors which occur here are difficult or impossible to check accurately in the editor's office. Careful attention on the part of the contributor in checking literature citations is appreciated.

We are now checking the abbreviations of Journal names with the standard list of journal abbreviations listed in the Index Catalog of the library of the Surgeon General's Office. We trust that this detail will show more uniformity in the future. Where this publication is available we would appreciate authors using it in abbreviating journal names.

Tabular and illustrative material—The arrangement of tabular material with more thought of its reproduction on the printed page is desirable. Tabular material should be arranged so that it can be set crosswise rather than lengthwise of the printed page. Authors, because of their familiarity with the data, can do this better than anyone else. In some instances we have rearranged tabular material so that this could be accomplished; in others we were unable to do so.

I am quite certain that large tables carrying excessive amounts of detailed material are discouraging to some readers and that tables summarizing these data are more effective. The investigator who is particularly interested in the details of such work will undoubtedly be in correspondence with the author concerning details.

There are, no doubt, many who would like to see greater uniformity of style in the articles carried in the Journal. This is particularly true in case of tables and illustrative materials. We realize that considerable flexibility must be allowed because of the variation in the data presented. At the same time there is ample room for improvement. Unfortunately we do not have a standard guide for our contributors to follow in preparing manuscripts for publication. If it is the opinion of the Association that a standard style brief for contributors to the Journal is desirable, there are two courses which we might follow. We might recommend one of the various publications now available as a standard for preparing manuscripts for our Journal, or a committee might be selected to prepare a brief style standard which could either be published in the Journal and reprinted for future distribution, or printed separately and distributed to our membership. I believe the latter plan would be preferable since it would provide an opportunity for giving specific recommendations, whereas most of the publications now available are more general in nature in order that they may be applied to a much broader scope of scientific subject matter than is carried by our Journal.

We appreciate the spirit of helpful cooperation accorded us the past year by those who have contributed to the Journal, by those who have given so generously of their time in reviewing articles, and by those who have called our attention to details which require more careful checking to avoid errors.''

Respectfully submitted,

T. S. SUTTON, *Editor*

Upon motion duly seconded, the report was accepted.

Mr. C. Y. Cannon presented the following report of the Committee on Resolutions:

RESOLUTIONS

WHEREAS during this, the thirty-fourth annual meeting of the American Dairy Science Association, its membership and their families have enjoyed the courteous hospitality of the University of Idaho and its sister institution, the Washington State College and the delightful entertainment and considerate care provided by members of their faculties, their wives, their students and their office help:

THEREFORE, Be it resolved:

That the secretary of the American Dairy Science Association be instructed to express by letters to the presidents of the University of Idaho and Washington State College the thanks of the Association for their courtesies and the use of their buildings and facilities.

WHEREAS the large part of the burden of arranging for registration, lodging, entertainment and transportation was carried by the faculty members of the Dairy Departments of the two schools with their wives:

THEREFORE, Be it resolved:

That the members of the American Dairy Science Association, their wives and families hereby express to these Dairy faculty members and their wives their grateful appreciation for the large effort this has cost them.

WHEREAS the cities of Moscow and Pullman provided free access to their parks, golf courses and swimming pools for the use of members of the Association and their families, and,

WHEREAS the Potlatch Forests, Inc. kindly permitted the wives and families of the members of the Association to visit under guided direction their lumber cutting plant at Lewiston, Idaho, and provided lunch for them:

THEREFORE, Be it resolved:

That the American Dairy Science Association express its appreciation to the cities of Moscow and Pullman and to the Potlatch Forests, Inc. for their kindnesses.

WHEREAS Dean E. V. Ellington and his Program Committee have done a tremendous amount of work in arranging the programs that have been presented before the various sections at this meeting:

THEREFORE, Be it resolved:

That the American Dairy Science Association extend its thanks to the chairman and his committee for their splendid and untiring efforts.

However, whereas it appears that an unusually large number of papers appearing in the printed program have not been presented because of the absence of the authors,

THEREFORE, Be it resolved:

That the members of the Association in the future exercise greater discretion in submitting titles and abstracts to the Program Committee when there is little likelihood of the authors' being able to attend the meeting, since it is believed that the abstracts should not be regarded merely as a convenient method of securing advance publication of research in progress.

WHEREAS the Borden Company is continuing its awards in recognition of superior research in the field of dairying:

THEREFORE, Be it resolved:

That the American Dairy Science Association express its appreciation to the Borden Company for its continued interest in dairy research.

WHEREAS Mr. W. A. Wentworth of the Borden Company whose gracious presentations of the Borden Awards in past years has added so greatly to our sessions, has suffered such gréat loss and personal injuries preventing his attendance at this session:

THEREFORE, Be it resolved:

That the Secretary of this Association be instructed to express to Mr. Wentworth, his family and business associates our great appreciation for his services to the American Dairy Science Association and to Mr. Wentworth our sincere sympathy, our deep regret that he could not be with us this year and our best wishes for his speedy and complete recovery.

WHEREAS the Extension Section of the American Dairy Science Association has formally adopted uniform rules governing the organization and conduct of Dairy Herd Improvement Associations, Standard Cooperative Bull Associations, Artificial Breeding Associations, and Bull Studs:

THEREFORE, Be it resolved:

That the American Dairy Science Association adopt and approve the action of the Extension Section pertaining to the above rules, copies of which are on file.

WHEREAS during the past year our highly esteemed associate and active supporter of the American Dairy Science Association, H. E. Van Norman, passed away, and,

WHEREAS this Association has sustained an irreparable loss in his passing:

THEREFORE, Be it resolved:

That the Association pause in its deliberations to pay honor to this man, and,

That the Secretary be instructed to transmit a copy of these resolutions to the family of the deceased.

Respectfully submitted,
C. Y. CANNON
D. R. THEOPHILUS
B. E. HORRALL
L. S. PALMER
OTTO HILL
Committee on Resolutions

Mr. I. R. Jones gave the following progress report on curricula in dairy production:

DAIRY PRODUCTION CURRICULUM SUB-COMMITTEE PROGRESS REPORT

The Committee has issued a questionnaire of 17 questions to its members and has summarized and exchanged the answers as a guide to developing its work. Other exchanges are now in progress within the sub-committee. Some of the things which seem to indicate the trend of the study to date are as follows:

1. Objectives of instruction in dairy production should be definitely determined. These objectives should be based upon a careful study of the conditions within a state and should specifically meet the problems of the dairy industry in that state. The answers to the questionnaires indicated a belief that many of the established courses will profit by continuous revision and modernization. The training should fit into and show the relationship with all phases of life in which the average graduate in dairy production must work.

2. The basic science or fundamental courses should be carefully studied with the idea of establishing some degree of standardization. This includes consideration of the question of prerequisites.

3. There should be ample opportunity for a student to elect courses outside the field of dairy husbandry.

4. Dairy manufacturing courses should be required of dairy production

students in order to enable them to obtain a broad understanding of the problems in the dairy manufacturing field.

5. The relationship of the dairy industry to the problems of sanitation and public health should form an important part of the training in dairy production.

6. The introductory as well as some of the important advanced courses should be organized so as to be equally useful to general students in agriculture and major students in dairy husbandry.

7. Individual work or honor courses seem to have a definite place for upper class students.

It is recommended that the committee be continued so that definite reports can be presented for adoption at a later date.

Respectfully submitted,
C. E. Wylie
J. W. Bartlett
C. N. Shepardson
T. E. Woodward
C. L. Clevenger
I. R. Jones, *Acting Chairman*

Upon motion duly seconded, the report was accepted.

Dr. C. L. Roadhouse then read the following report from the sub-committee for dairy manufacturing curricula:

REPORT OF SUB-COMMITTEE ON DAIRY MANUFACTURES CURRICULUM

AMERICAN DAIRY SCIENCE ASSOCIATION

JUNE, 1939

A study has been made of 25 dairy manufacturing curricula in colleges where dairy manufactures is taught as a major subject. The study was greatly aided by the availability of material prepared by H. B. Ellenberger, Chairman of the general committee. It has been noted that there is a great variation in the number of units required for graduation and a surprising variation in the number of units required in basic sciences, such as chemistry, physics, mathematics, bacteriology, zoology and botany. In four leading colleges the number of units required for graduation varied from 120 to 155 and the number of units of work in basic sciences varied from 18 to 50.

The committee has been interested primarily in the number of units required in basic sciences for graduates in dairy manufacturing. The majority of the members have favored a standardization of dairy manufacturing curricula in agricultural colleges to give a basic training to students and a good general education. This is especially desirable for students who undertake graduate study in institutions other than the one in which they receive their B.S. degree.

Two points of view have been expressed concerning the curriculum in dairy manufactures.

(1) That there should be a separate curriculum for students specializing in dairy manufactures.

(2) That there should be one curriculum that would serve both the manufacturing and production fields of dairying in basic training and allow a liberal choice of electives to serve the needs of students specializing in the separate fields.

The view has been expressed that all dairy students should be required to complete courses in animal physiology, nutrition, feeding, milk production and principles of dairying and such additional courses in dairying as may be of interest to them. The manufacturing and production groups should then be required to complete all of the course offered in their respective fields.

Several points of view have been expressed by the committee and other members of the American Dairy Science Association. Further study will be required to complete a final report, but from the consideration of the subject by the present committee, it is apparent that a unification of the curricula in the various colleges would be very desirable.

R. W. Bell
A. D. Burke
C. D. Dahle
J. H. Erb
H. F. Judkins
W. V. Price
C. L. Roadhouse (*Chairman*)

Upon motion duly seconded the report was accepted.

Mr. Otto J. Hill, Secretary of the Extension Section, read the following report:

EXTENSION SECTION REPORT

The annual business meeting of the Extension Section was called to order by S. J. Brownell, Chairman, June 28 at 9:30 A.M. in the U. C. B. Building, Moscow, Idaho, with 53 attending.

During the two-day session the papers and committee reports were presented as given in the program. The sire committee report was presented by Floyd Arnold. This report was accepted with slight changes in wording.

The type classification committee report was given by A. C. Baltzer. Motion was made by G. E. Gordon and seconded by G. E. Vergeront as follows: The Extension Section advises the type classification committee to work in close cooperation with the breed relations committee during the coming year.

Election of Officers. Nominating Committee: E. J. Perry, C. L. Blackman, Ivan Loughary. Two nominees were presented, Warren Gifford and Glen Vergeront. Vergeront was elected secretary. R. G. Connelly automatically becomes chairman and O. J. Hill, vice-chairman. Motion was made by Blackman and seconded by Taylor that hereafter the number of sessions in the Extension Section be so arranged that at least one joint meeting could be held with the Production Section. The joint sessions should be of interest to both. Motion carried.

The following motion introduced by A. R. Merrill was carried:

> "Because of the increased interest in the work of the College Feed Conference Board on the part of dairy workers in the Southeast and on the Pacific Coast, I move that the Chairman of the Production and Extension Sections of the American Dairy Science Association appoint a joint committee of not less than five to make a study of the work of the Board and report at the next annual meeting on the advisability of including this work in the work of the Association. If the committee should decide that it is advisable to include the work of the Board within the Association it is asked to bring in tentative plans for its organization as a working part of the Association."

The meeting was then adjourned.

Respectfully submitted,
O. J. HILL,
Secretary.

Upon motion duly seconded, the report was accepted.

Mr. P. A. Downs, chairman of the Manufacturing Section, read the following report:

MANUFACTURING SECTION REPORT

The Manufacturing Section held its regular meetings at the appointed hour and place. Dr. P. A. Downs presided. The papers presented developed a well-balanced program which held the interest of the group as evidenced by the discussions when time permitted.

The meetings were conducted with dispatch and promptness and adjourned at scheduled time.

All papers were presented as scheduled except M1, M2, M5, M10, M29 and M37 which were read by number.

Dr. Downs appointed a nominating committee consisting of: G. M. Trout and C. L. Roadhouse, to nominate a vice-chairman and a secretary for the coming year.

Old Business:—Report of Committees

1. Committee on Chemical Methods for the Analysis of Milk and Dairy Products. G. H. Wilster reported for Prof. Thomson, chairman, a written report. Report accepted and filed.

2. Committee on Quality Program. P. A. Downs presented a lengthy progress report, submitted by W. H. E. Reid, Chairman. Report accepted and filed.

3. Committee on Judging of Dairy Products. Report submitted by G. M. Trout, Chairman. Report accepted and filed.

4. Committee on Methods of Determining the Curd Tension of Milk. A progress report submitted by L. A. Chambers was read. Report accepted and filed.

5. Committee on Score Cards for Sanitary Inspection of Dairy Farms. Report read by C. L. Roadhouse. Report accepted and filed.

6. Committee to Study Methods for Measuring the Oxidation of Milk Fat. Report of progress read by Dr. Garrett, chairman. Report accepted and filed.

7. Method for the Bacteriological Analysis of Milk and Dairy Products. Progress report read by Dr. Macy, Chairman. Report accepted and filed.

8. Committee on Methods of Measuring the Color of Milk. Progress report read by Professor Garrett, chairman. Report accepted and filed.

9. Committee on Sanitary Procedure. Professor L. H. Burgwald, Chairman. No members of committee present and report not submitted.

New Business:—

A discussion was held regarding the desirability of a possible change in the method of presentation of papers.

It was moved that a committee of three be appointed to work with the Manufacturing Program Committee to draw up possible changes in the program arrangement. Committee to be appointed by section chairman. Motion carried.

The nomination committee placed the following names in nomination for officers for the year October 1, 1939, to September 30, 1940.

Vice-chairman: C. D. Dahle, K. M. Renner.

Secretary: S. T. Coulter, R. Whitaker.

A vote by ballot was taken, and the secretary with C. L. Roadhouse and G. M. Trout acted as tellers.

The following officers were elected:

Vice-chairman: C. D. Dahle.

Secretary: S. T. Coulter.

Business session adjourned.

Respectfully submitted,
T. H. HERZER, *Vice-chairman,*
Acting Secretary of Manufacturing Section.

Upon motion duly seconded, the report was accepted.

Mr. H. A. Herman, acting secretary of the Production Section, then submitted the following report:

PRODUCTION SECTION REPORT

The Production Section held four sessions at the regularly scheduled hours and places with the Section Chairman, H. W. Cave, presiding at the opening session on milk secretion, physiology and udder diseases; A. H. Kuhlman, chairman for the pasture, hay and silage session; I. W. Rupel, chairman for the nutrition session, and H. W. Cave, chairman for the final session on Thursday afternoon, June 29th.

All sessions were well attended and considerable interest was shown in the papers presented. The papers were well prepared and very ably presented. Slides, charts and mimeographed material were used to a good advantage in many of the presentations. All but six of the forty-one papers listed on the program were presented. The printed program indicates the member who presented each paper.

The business meeting of the session of the Production Section was held at 3:30 Thursday afternoon, June 29, with 41 members in attendance. H. W. Cave, Chairman, presided and in the absence of A. L. Beam, H. A. Herman was appointed to serve as secretary for the meeting. The minutes of the 1938 meeting at Columbus, Ohio, were read and approved.

Reports were submitted by the various standing committees and approved. Copies of these reports are being placed on file.

Points of particular interest incorporated in the adopted reports are:

Breeds Relation Committee, J. W. Bartlett, Chairman (presented by H. A. Herman):

1. In cooperation with the dairy breed association officers, this committee has worked out a plan of registering offspring resulting from artificial breeding of dairy cows and has prepared a recommended service form for this purpose, as follows:—

(IMPORTANT) This form must be forwarded to the breed association within three days after the cow is inseminated. A certificate must be sent in for each insemination. (Not required where the owner of the bull is the owner of the inseminated cow.)

CERTIFICATE OF COLLECTION OF SEMEN

This is to certify that .... took semen from the bull
.... Herd Book No. .... Owned by ....
on date .... This semen was placed in a sealed container marked as follows:

....
....
and shipped or delivered to ....
....

(SIGNED) ....
(Owner or authorized agent)

Address ....

CERTIFICATE OF ARTIFICIAL INSEMINATION

This is to certify that .... inseminated the female
.... Herd Book No. .... owned by ....
with semen from container marked as follows: .... and certified
to be from sire (Name & No.) .... on date ....
(SIGNED) ....
(Owner or authorized agent)
Address ....
Signature of witness of Insemination ....

(Owner of the Service Sire may insert name of the cow for which the semen is to be used.)

2. It was deemed unwise to make further additions to the testing rules concerning thyroxine administration so as to include certain placental extracts and cocoa meal.

3. It is recommended that it be left to the discretion of the production testing department of the breed associations as to whether surprise tests be *in addition to* or supplant the regular test.

4. The committee desires to call to the attention of the production section, as well as the respective state superintendents of official testing, that obligatory herd test retest requirements exist and the present wording of the uniform rules for herd improvement registry specifies conditions under which they apply.

5. It is recommended that the uniform rules for the herd improvement registry test concerning surprise tests be amended so as to read "one or more surprise tests with a preliminary milking *may* be made by a different supervisor during the testing year."

6. Regarding the mixing of milk for sampling purposes in the conduct of official tests, the committee feels that thorough mixing of milk is accomplished when the milk is poured from pail to pail several times.

Report of Committee on Methods of Measuring Results of Pasture Investigations, R. H. Lush, Chairman (presented by I. R. Jones).

It was recommended:

1. A further study and a revision of the 1936 report on pasture investigations as presented by a joint committee of the American Dairy Science Association, the American Society of Agronomy, and the American Society of Animal Production be made this next year.

2. It is recommended that this association committee cooperate with the American Society of Agronomy and the American Society of Animal Production and that any reports adopted be published in scientific journals of the societies in question rather than other agencies.

Committee on Standard Methods, A. E. Perkins, Chairman (presented by W. E. Peterson).

Report as prepared by Dr. C. F. Huffman on Feed and Feces Analyses presented and discussed. In view of no further reports and problems involved in presenting the extensive mass of material to the membership, it was voted to continue the committee another year in order to complete the work now under way.

Committee on Rules for Conduct of the Students National Dairy Cattle Judging Contest, I. W. Rupel, Chairman.

It was agreed that:

1. There would be no material changes made at this time in the rules for conduct of the contest.

2. At the request of Mr. Oderkirk, superintendent, it was agreed that a greater portion of the work of compilation and tabulation of contest results could be handled by the coaches.

Committee on Awards for Students National Judging Contest, A. A. Borland, Chairman.

It was recommended:

1. That the attempt to secure more scholarships for winners in the Students' National Contest be continued and the present committee continue its effort in this direction.

Dr. E. S. Savage, Cornell, introduced a motion that the chairman of the Production Section appoint a joint committee of not less than five to make a study of the work of the College Feed Conference Board and report at the next annual meeting. If the committee should decide, in view of the interest of the work of the Board, that it should be a working part of the Association that plans be inaugurated to make this feasible. Motion seconded and carried. This committee will be appointed later.

All standing committees were reappointed with their present personnel except as follows:

Breeds Relation Committee: F. W. Atkeson, Kansas, and Floyd Johnston, Iowa, were appointed for a period of three years to replace C. E. Wylie and J. G. Hays whose terms expired.

Committee on Methods of Measuring Results of Pasture Investigations changed as follows:

G. BOHSTEDT, Chairman
R. H. LUSH
I. R. JONES
R. E. HODGSON
C. E. BENDER
R. B. BECKER

Committee on Standard Methods: C. H. Whitnah, Kansas, appointed on Milk Analyses to succeed A. E. Perkins. Other members remain the same, with W. E. Peterson as general chairman of the committee.

G. Bohstedt, chairman of the nominating Committee presented candidates for the offices of vice-chairman and secretary for 1940. W. E. Peterson of Minnesota was elected vice-chairman and H. A. Herman, Missouri, was elected secretary. A. H. Kuhlman, vice-chairman for this year, automatically becomes chairman for 1940.

Respectfully submitted,
H. W. CAVE, *Chairman*
H. A. HERMAN, *Acting Secretary*

The Nominating Committee submits the names of the following men as officers of the Association: For Vice-President, Professor Harry Cave, Kansas State College; Professor C. Y. Cannon, Iowa State College.

For Directors, Charles N. Shepardson, Texas A. & M.; H. O. Henderson, University of West Virginia.

Fordyce Ely, University of Kentucky; J. W. Bartlett, University of New Jersey.

Respectfully submitted,

O. E. Reed
C. A. Smith
I. R. Jones
P. S. Lucas

Mr. C. C. Prouty and Mr. Henry Hanson submitted the following attendance report:

	Members	*Non-Members*	*Ladies*	*Children*	*Total*
Dist. of Columbia	5	0	3	3	11
Arizona	2	0	2	0	4
California	9	1	6	0	16
Connecticut	1	0	1	0	2
Idaho	6	4	5	5	20
Illinois	4	0	3	0	7
Indiana	2	0	2	1	5
Iowa	5	0	4	3	12
Kansas	6	0	3	2	11
Kentucky	1	0	1	2	4
Maryland	2	0	1	0	3
Massachusetts	2	0	2	1	5
Michigan	5	2	6	9	22
Minnesota	7	0	6	10	23
Mississippi	2	0	3	1	6
Missouri	8	0	4	4	16
Montana	2	0	2	1	5
Nebraska	2	0	2	1	5
Nevada	1	0	0	0	1
New Jersey	2	0	1	0	3
New York	4	0	3	2	9
Ohio	9	0	8	8	25
Oklahoma	1	0	0	0	1
Oregon	10	1	4	0	15
Pennsylvania	3	1	2	2	8
So. Carolina	2	0	1	0	3
So. Dakota	2	0	2	3	7
Texas	2	0	2	2	6
Utah	5	1	2	0	8
Vermont	1	0	1	1	3
Washington	25	23	22	12	82
West Virginia	1	0	1	2	4
Wisconsin	5	0	3	3	11
Wyoming	1	0	1	3	5
Canada	8	9	3	0	20
New Zealand	0	1	1	0	2
	151	45	113	81	390

American Dairy Science Association Presented Borden Awards to S. L. Tuckey and R. E. Hodgson

at the

ANNUAL BANQUET
Washington Hotel
Pullman, Washington, June 29, 1939

E. V. Ellington, toastmaster, presented the officers and distinguished guests. He then introduced P. A. Downs, chairman of the Dairy Manufacturing Section, who made the following statement:

"According to the rules adopted by the Executive Board of the American Dairy Science Association, the Borden Award in Dairy Manufactures is awarded for outstanding research in this field. The recipient must not have reached the end of his 40th year and must have published the results of meritorious research during the preceding five-year period.

The members of the Dairy Manufacturing Award Committee of this Association have given careful consideration to all those recommended by the Nominating Committee and have unanimously chosen Dr. S. L. Tuckey to be thus honored this year.

Dr. Tuckey was selected for the honor on the contribution which he has made in the application of the X-ray diffraction analysis of dairy products.

Dr. Tuckey was born in Brown's Valley, Minnesota, August 24, 1905 and later moved to Peoria, Illinois. He received his undergraduate work at the Bradley Polytechnic Institute at Peoria and the University of Illinois where he received his Bachelor of Science degree in 1928. He was also granted the Master of Science degree in Dairy Husbandry in 1930 and the degree of Doctor of Philosophy in 1937 from the same institution.

Dr. Tuckey has contributed much to our knowledge of the crystalline structure of milk proteins and the process of cheese ripening, thus helping to supply a new tool for research workers in the dairy field. The results of his research have appeared in many articles published both in scientific journals and trade papers. He has brought honor to himself and to the institution of which he is a member through his efforts to serve the industry.

Mr. Foster, it is now my pleasure to present to you as the recipient of the Borden Award in Dairy Manufacturing, Dr. S. L. Tuckey."

"Dr. Tuckey, on behalf of the Borden Company and the 30,000 men and women who daily work with that organization, it gives me a lot of pleasure to extend to you the gold award under the direction of the American Dairy Science Association, for outstanding achievement in dairy manufacturing research.

My one regret is that Mr. Wentworth couldn't be here to present it as he has in the years gone by, but I know I present his sentiments and the heartiest congratulations of all the men in the Borden Company when I give you

S. L. TUCKEY

this gold award, which is the lasting memento of your accomplishments. And with it goes the paper award with which we hope you may purchase, and acquire, and enjoy something you have been wanting for a long, long while."

The toastmaster then introduced H. W. Cave, chairman of the Production Section, who made the following statement:

"The Dairy Production Award Committee of the American Dairy Science Association has the function of selecting the person to receive the Borden Award in Dairy Production in accordance with the rules adopted by the Executive Board of the Association. The award this year is conferred for meritorious research in the field of dairy production, the results of which have been published during the preceding five-year period by a person who has not yet reached the end of his fortieth year.

The three members of this committee, individually, gave careful consideration to all who had been recommended by the Nominating Committee and as a result have unanimously chosen Mr. R. E. Hodgson, Agent, Bureau of Dairy Industry, United States Department of Agriculture, and Dairy Husbandman, Western Washington Experiment Station, Puyallup, Washington, to be thus honored this year in consideration of the important and able investigations he has reported.

Mr. Hodgson is selected for this honor primarily because of the extensive investigations in which he has been the leader and the resultlant valuable contributions to our knowledge of the nutrient values of herbage of different kinds and their utilization by dairy cattle. The findings from researches conducted by Mr. Hodgson and his co-workers, including Knott, Murer, Ellington and Graves, have both scientific significance and practical application in relation to problems which confront the industry.

Mr. Hodgson assisted by his colleagues has made searching studies pertaining to: Factors affecting and methods of measuring pasture yields; factors affecting the availability of nutrients in pasture herbage and other roughages; methods of preserving and of determining nutrient values of various roughages; calcifying properties and milk producing values of green and dried pasture herbage, the content and utilization of carotene and vitamin A in different roughages; and the nutritive value of home-grown roughage rations for dairy cattle.

The Award Committee is pleased to present Mr. Hodgson to receive the Borden Award in Dairy Production for 1939.

Mr. Hodgson, will you please come forward to receive your award. Mr. Foster, I would like to present to you Mr. R. E. Hodgson for the award in Dairy Production."

Mr. Foster then spoke, "Mr. Hodgson, it is a distinct pleasure to welcome a prophet in his own home town and to give you on behalf of the Borden Company as a symbol of your achievement in the field of Dairy Production this lasting gold medal known as the Borden Award.

R. E. HODGSON

The production of milk and the caring for or feeding dairy cattle eventually reflects in a better economic status of the producers of milk and it must always be of greatest interest to the people of our industry, so we present to you with our company's compliments and best wishes the gold symbol and likewise the paper which means a great deal.

The Borden Company congratulates you and wishes you further success in your field of chosen endeavor. May this financial recognition of your unselfish work enable you to acquire something you have wanted but have not enjoyed. Thank you."

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THE EFFECT OF ADVANCED LACTATION IN THE COW ON THE PHYSICAL AND CHEMICAL COMPOSITION OF THE MILK

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In herds under bacteriological supervision where it is the practice to milk cows longer than seven or eight months following parturition, it has been found that physical and chemical changes occur in the milk of certain animals and that these changes are usually more common to all quarters. The object of this paper is to show that these characteristic changes in the composition of the secretion during advanced lactation in many instances are probably produced by causes other than bacterial infections.

In the literature pertaining to mastitis there are very few data concerning this phenomenon, or the results of periodic laboratory examinations of milk during the complete span of a lactation period. Johns (1), Johns and Hastings (2), and Hastings and Beach (3) reported the results of the examination of the milk of young cows (complete lactations) free from streptococci by the use of indirect chemical tests and bacteriological methods. In a group of 31 young cows studied during their first lactation periods, Hastings and Beach (3) found that 14 persistently yielded abnormal milk, occasionally from only a single quarter. Klein *et al.* (4, 5) showed that milk samples from noninfected cows in the intermediate stage of lactation had a lower pH and chlorine concentration than the milk from cows in either the earlier or the later stage of lactation. They reported (5) that the high values in pH, chlorine content, and leucocyte counts in a group of special cows probably were associated with advanced lactation. Plastridge (6), Edwards (7), and Udall and Johnson (8) found that the milk from uninfected cows during the later months of milking or when the cows began to dry off gave higher pH determinations than the milk in their earlier lactation periods. These observations have been partly confirmed by a different approach in dairy research primarily concerned with the physical and chemical changes occurring in the milk as the result of prolonged milking periods. It has been reported that the acidity (9), the chlorine content (10), and the curd tension (11) of the milk may be increased during advanced lactation. Furthermore, especially in pregnant cows, there occurs a more marked decrease of

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the milk flow (12) during the later period of milking. This decrease in the milk yield is accompanied by an increase in the percentage of fat (13), a reduction (14) in the size of the fat globules, and the development of a bitter taste attributed to the secretion of a fat lipase (15) which also interferes (14) with the churning ability of the cream.

The significance of these chemical and physical changes of the milk as related to prolonged milking has been briefly discussed by Espe in his recent book "Secretion of Milk" (16). He says: "With the increasing percentage of fat in the milk commonly associated with advancing lactation there also occur other characteristic changes, including an increase of most of the milk solids except lactose. Occasionally the salt content of milk secreted at this time tends to increase markedly; causing certain investigators to suggest that after several months' lactation the composition of the milk tends to approach the composition of blood. They do not mean to infer that the organized bodies in blood, such as the red and white corpuscles, appear but that instead of the epithelial cells of the alveoli synthesizing the constituents occurring in normal milk, the normal selective absorption of the semi-permeable cell membrane is reduced, permitting certain constituents of the blood to pass into the milk unchanged. In fact, it has even been suggested that serum globulin and possibly albumin may appear in the milk in increasing amounts at this time. In addition to a sharp rise in chloride content there is an increase of pH, as well as enzyme and catalase values."

From the literature just cited it is not clear at what period of milking the cow is considered to be in advanced lactation. It has been reported, however, that the acidity (9) remains fairly constant from the second through the seventh month; the fat (13) increases after 7 or 8 months of milking, and the effect of pregnancy (12) becomes apparent by a reduction of the milk yield after a five months' gestation period. Hence, if a cow conceives to a service 2 months following parturition, these changes (increase in pH, percentage of chlorides, and leucocyte count) may be expected to occur in the milk of certain cows after a lactation period of 7 or 8 months' duration. Thus after this time the animal may be considered to be in advanced lactation.

MATERIAL

The data for this publication were obtained from three widely separated herds in which an attempt has been made to control mastitis by the monthly laboratory examination of the fore milk. As originally planned, this study was to be continued indefinitely, so the present observation was merely incidental to the main objective. The herds have been under observation for the following periods: herd A for 36 months, and herds B and C for 26 months. In herd A all the cows infected with Group I streptococci (*Streptococcus agalactiae*) (17) were eliminated, whereas in herd B they were quarantined in a separate barn, and in herd C they were segregated in the same barn with the normal cows but were milked last. In each herd special precautions were employed in the stable management, and the milking operation was carefully supervised.

In this study only those cows were selected in which the milk failed to show the presence of mastitis streptococci or alteration due to staphylococci or miscellaneous organisms.

METHODS

When a bacteriological examination of the milk was made, the preliminary laboratory work was conducted at the respective farms. The routine methods employed have already been reported in detail (18). The samples of the fore milk (3 or 4 cc.) upon collection were kept chilled, and 1 cc. of a 1:10 dilution of it was plated in blood agar. The leucocyte films also were prepared on the premises and the chloride dilutions set up.

RESULTS OF THE LABORATORY EXAMINATION OF THE MILK

In Table 1, under section A, are given the results of the laboratory examination of the milk from 112 quarters of 24 cows conducted during the first four months of lactation. Section B of this table includes the results of the laboratory examination of the milk from these same cows after lactation

TABLE 1

Laboratory tests of samples of milk from cows, (A) at the beginning, and (B) at the end of the same lactation periods, and (C) at the beginning of the succeeding lactation periods

No. of cows	No. of quarters	pH			Percentage of chlorides		Leucocyte count			Bacteria count				
		6.6	6.7	6.8 and over	Under 0.140 per cent	Over 0.140 per cent	0 to 300,000	300,000 to 1,000,000	1,000,000 and over	10 to 3,000	3,000 to 10,000	10,000 to 20,000	20,000 to 40,000	40,000 to 100,000
(A) During first 4 months of lactation														
24*	112	109	2	1	101	11	108	4		97	13	.	2	
Percentage		97.3	1.8	0.9	90.0	9.8	96.4	3.6	. .	86.6	11.6		1.8	.
(B) After 8 months or more of lactation														
24*	112	41	47	24	15	97	64	36	12	47	31	21	11	2
Percentage		36.6	42.0	21.4	13.4	86.0	57.2	32.2	10.7	42	27.7	18.7	9.9	1.8
(C) At beginning of the succeeding lactation														
16**	64	53	10	1	52	12	60	4	...	47	11	5	1	...
Percentage		82.8	15.6	1.6	81.2	18.7	93.8	6.2	. .	73.4	17.2	7.8	1.6	. .

* The milk of 4 of the 24 cows was examined through two complete lactations.

** 8 cows were not available for examination at this lactation, since 4 had been sold, 2 had died, and 2 had not yet calved.

periods of 8 months or longer. Under section C are presented the results of the laboratory examination of the milk from 64 quarters of 16 of these cows at the beginning of the succeeding lactations.

It will be noted in Table 1 under section A that the majority of the samples showed a pH of 6.6 and that the majority of the chloride determinations were not above 0.14 per cent. In 96 per cent of the samples the leucocyte counts (neutrophils) were not over 300,000 cells per cc. of milk. Under section B, however, it is shown that approximately 63 per cent of the samples gave a pH reading of 6.7 or above, while in 86 per cent the chloride determinations were above 0.14 per cent. In 32 per cent of the samples the leucocyte count varied between 300,000 and 1,000,000 cells per cc. (neutrophils), but in 10 per cent it was above 1,000,000. In the latter group the bacterial counts were correspondingly higher, and the predominating microorganisms were staphylococci (other than *Staphylococcus aureus*) and diphtheroids. However, the milk from 15 other cows in the three herds, with bacteria counts nearly as high in the late stage of lactation, showed very little change in pH, percentage of chlorides, and the leucocyte count. This fact is evidence that high bacteria counts and the physical and chemical changes under discussion are not necessarily associated with each other. Hence it may be assumed that these changes are not always caused by bacterial infections. Support for this belief is provided in the observation that usually, in udders infected with staphylococci and miscellaneous microorganisms, not all quarters show these characteristic changes. In this series of cases, however, the animals included in the table usually showed more uniform cell counts and changes in the chemical composition of the milk from all quarters.

It is significant, as noted under section C, that in the early part of the succeeding lactations some of the cows produced a milk of lower quality than that of their previous lactations (increase in pH and percentage of chlorides), thus indicating some impairment of the udder which occurred during the previous lactation. It should be mentioned that 4 cows developed mild cases of mastitis caused by Group III streptococci (17) (*Str. uberis*) 3, 4, 6, and 8 months respectively after the last bacteriological examination of the milk reported in Table 1, under section C.

DISCUSSION

The results of the laboratory examination of the fore milk from 112 quarters of 24 uninfected cows in their late lactation periods, as compared with the determinations from these cows in the early stage of the same lactations, showed a marked increase in alkalinity, percentage of chlorides, number of leucocytes, and, occasionally, bacteria counts. This change in the reaction of the milk during prolonged milking was not common to all the cows in the three herds. Usually it was more noticeable in the milk from the older animals, but the same phenomenon was observed in the milk from 10 first-calf heifers. The change in the character of the secretion from some cows during prolonged milking may plausibly be explained by the theory that the lining alveoli in certain udders were more permeable to the constituents of the blood. It should be mentioned here that a majority of the determinations (section B) were obtained from cows during periods of equable weather, thus eliminating extreme climatic changes as contributory factors responsible for the abnormalities of the milk obtained in advanced lactations.

The literature (9–16) suggests that in advanced lactation the physical and chemical composition of the secretion becomes altered so that the milk produced at this time perhaps may be regarded as abnormal. This alteration in the normal constituents of the secretion may in some way influence the effectiveness of the inhibitory or bacteriostatic substance present in normal milk which is primarily concerned in the protection (19–22) of the udder. In other words, if the normal protective mechanism of the milk or udder is affected, the common udder flora or the more infective types of streptococci or staphylococci should develop more readily. On the other hand if the potency of the inhibitor remains unchanged the increased permeability of the cell membrane may allow the passage of the constituents of the blood in certain cows to reach the udder unchanged and thereby supply nutritive material which renders the environment more favorable for bacterial growth. Thus it is likely that animals in advanced lactation, due to the changes in the character of the secretion, may be more prone to infection or to an increase in the general bacterial population of the udder than cows milked for shorter periods. Furthermore, the slight increase in the leucocyte count in addition to the change in the physical and chemical composition of the secretion suggests that prolonged milking may also be responsible for the induration found in the udders in which there is no history of mastitis.

In the study of the pathology of "nonspecific mastitis" Peterson and his associates (23) reported chemical and physical changes occurring in the udders of young cows during one or more lactations which were not attributed to streptococci or the more infectious types of staphylococci. They suggested, therefore, that nonspecific mastitis might be caused by an unknown agent, possibly a filtrable virus. Before nonspecific mastitis can be attributed to some special etiological agent, other factors—including the effect of advanced lactation—should be carefully considered. Such a study should include the effect, if any, of heredity on the permeability of the udder; the effect on the udder of hand versus machine milking; the effect on the udder of a forced high milk yield as compared to a more normal yield, and a controlled study under normal conditions of the effect of the physiological secretion of milk in conjunction with a thorough survey of the bacteriological population of the udders of the cows studied.

In regard to the data here presented, it remains for future observations to determine if it is a better practice for the dairyman to shorten the lactation period of the cow in an attempt to preserve her udder, or to continue the milking into advanced lactations, with little consideration for the general health of the cow and the unborn calf.

SUMMARY

1. The results are given of the laboratory examination of the milk from 112 quarters of 24 cows during the early and late stages of their lactation periods.

2. It is shown that the milk produced by certain cows after 8 months of lactation had a higher pH, percentage of chlorides, and cell content than the milk the same cows had produced during the first 4 months of the same or succeeding lactation periods.

3. In view of these observations it is suggested that prolonged milking is conducive to certain physical and chemical changes in the secretion which affect the normal bacteriostatic substance in the milk. These changes, perhaps, not only increase the susceptibility of the gland to bacterial multiplication but also may result in a low-grade physical change in the secretory tissue of the udder and eventually shorten the total production life of the cow.

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FACTORS AFFECTING THE PASSAGE OF LIQUIDS INTO THE RUMEN OF THE DAIRY CALF. I. METHOD OF ADMINISTERING LIQUIDS: DRINKING FROM OPEN PAIL VERSUS SUCKING THROUGH A RUBBER NIPPLE[1]

GEORGE H. WISE* AND G. W. ANDERSON*

Dairy Husbandry Department, South Carolina Agricultural Experiment Station, Clemson, South Carolina

INTRODUCTION

The unthrifty appearance commonly observed in young dairy calves in modern dairy herds has been attributed, in many cases, to the deviation from "nature's way" of feeding. As substantiating etiological evidence, attention frequently is called to the fact that calves permitted to nurse cows are generally free of ailments usually observed in calves fed from open pails.

The thriftiness of calves permitted to remain with a nurse-cow may be ascribed to several factors, namely: the frequent consumption of relatively small amounts of milk; ingestion of milk at a constant temperature; the intake of gases that are normally present in the milk in the udder; slow consumption of the milk permitting much saliva to be mixed with it; and the passage of the milk directly to the abomasum. This last factor is the basis for much exploitation by manufacturers of certain calf feeds and calf feeders.

The contention is that when calves drink from pails, the milk is forced into the rumen where it undergoes abnormal decomposition causing physiological disturbances. In view of the paucity of experimental evidence this investigation was designed primarily to ascertain the extent to which pail feeding permitted and sucking prevented the passage of milk and other liquids into the rumens of calves.

REVIEW OF LITERATURE

Several calf feeders have been devised and used in an effort to simulate the natural method of milk consumption. Nearly all of them have a rubber nipple of some type. Several investigators (4) (6) (13) (15) reported that feeding calves with a good artificial feeder produced better growth and development than did feeding from pails. There is, however, little definite fundamental physiological information that may explain the observed results.

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From a study of the functional importance of the esophageal groove of young calves Schalk and Amadon (8) reported results that throw some light on the problem. By means of gastric fistulae in three calves about ten days of age, these investigators determined the course followed by the milk when it was drunk from a pail and when it was sucked through a nipple made from rubber finger cots. When the milk was taken through a nipple, none except a spurt as nursing began escaped into the rumen. Thus it was concluded that the liquid was coursing through the esophageal groove directly into the omasum and abomasum. On the contrary, during the act of drinking, the major portion of the milk ingested escaped from the proximal extremity of the groove into the rumen and reticulum.

Confirmatory evidence is available from work with other ruminants. It was observed that in suckling kids (1) (11) (12) and lambs (5), milk and other liquids traversed the esophageal groove to the abomasum. However, regurgitation of some of the material into the reticulum and rumen occurred within one hour after consumption.

According to Dukes (2) the groove of the young calf functions also as a conduit for water during the first few weeks of life. After that time, the response of the esophageal groove to water begins to wane permitting it to enter the rumen and reticulum.

EXPERIMENTAL METHODS

Two systems of feeding, (a) the common open pail from which the calf drinks the milk and (b) a nipple calf feeder,* the essential feature of which is a valved rubber nipple through which the calf sucks the milk, were used in this investigation. In the preliminary phases of this work each calf was fed by one of the methods from three days to 10 or 14 days of age, when it was slaughtered. Pasteurized milk from a mixed herd of Guernseys, Jerseys and Holsteins was fed. The daily allotment, approximately 10 per cent of the body weight of the calf, was given in two feeds, morning and evening.

The methods employed in determining whether or not the liquid entered the rumen and reticulum were also twofold. In the preliminary work each calf was slaughtered and the stomach contents examined. The last feed of milk was stained a brilliant green with a coal tar dye** to facilitate identification in the stomach. Precautions were taken in felling the calf, in segregating the compartments of its stomach and in excising the stomach to prevent the possible transfer of milk from one section to another. The obvious disadvantages of this technique necessitated adoption of a more satisfactory method.

* Coyner feeder pail, sold by Armour and Company, U. S. Yards, Chicago, Ill.

** Food coloring manufactured by Blanke-Baer Extract & Preserving Co., St. Louis, Mo.

The second and preferable procedure used was observation through rumen fistulae. These were established in seven male calves (Table 1). Since the surgical technique employed was somewhat different from that generally described, the procedure is presented in detail as follows:

The operative area on the left side was clipped, shaved, washed and painted with iodine. A local anesthetic, two per cent procaine solution, was injected along the line of incision located midway between the tuber coxae of the ilium and the vertebral attachment of the last thoracic vertebra. The incision, directed downward and slightly forward, was oval in shape, two to three inches in length, and started as near as possible to the transverse process of the fourth lumbar vertebra. The skin, fascia, muscles and peritoneum were incised and spread open. The rumen wall was then picked up and a portion of it was attached to the edges of the skin with interrupted linen sutures. The center portion of the sutured rumen protruded and separated the wound edges of the skin thus preventing their healing and closing the opening. As soon as the rumen and skin edges had firmly united, which occurred in four to five days, the sutures were removed and the rumen was opened.

The wound was washed daily with an antiseptic solution followed by an application of urea wound dusting powder (urea with Potassium-Oxy-Quinolin Sulphate) on all the wound surfaces until complete healing had occurred.

In one calf a transverse instead of a vertical fistula was made. It was

FIG. 1. Observation through the transverse fistula of an experimental calf.

four inches in length, and located as near as possible to the transverse processes of the lumbar vertebrae and just above the upper posterior border of the obliquus abdomnis externis muscle. The surgical procedure and the post operative care was the same as that used on the other calves. This method seemed to be more satisfactory as there was less discharge of the rumen contents through the fistula.

Observations were made by direct visual inspection. The interior of the rumen was illuminated by means of a diagnostic light which is small enough for convenient manipulation within the cavity. Inspections were made, as shown in Figure 1, while the calf was consuming the liquid.

As in the preliminary investigations, the fistulated calves were fed pasteurized, mixed herd, milk twice daily. The systems of feeding the milk were alternated from time to time throughout the duration of the experiment. The number of subjects and periods of observations are indicated in Table 1. During the early stages of the work, only milk and water, were given. Later, however, grain and hay were fed.

TABLE 1

Period of observation and initial systems of feeding fistulated calves

Herd No.	Breed	Age		Initial feeding method
		Started	Concluded	
		Days	*Days*	
C–29	Holstein	57	164	Pail
C–30	Holstein	29	94	Pail
C–32	Holstein	23	155	Nipple
C–34	Holstein	22	129	Pail
C–36	Holstein	19	126	Pail
C–37	Holstein	17	107	Nipple
349–B	Jersey	33	176	Pail

EXPERIMENTAL RESULTS

The preliminary results from post-mortem observations, shown in Table 2, indicate that when the milk was taken through a nipple by the young calf, none of it could be detected in either the rumen or the reticulum. When the milk was drunk from an open pail, in several cases some of it escaped into the rumen. Subjects E–3 and E–4 revealed no evidence of any milk in the first two compartments of the stomach. The amount of milk found in the rumen and the reticulum of calves E–1 and E–2 was a very small proportion of the total quantity consumed. In all cases a small amount of curd was observed in the omasum. The greatest amount of the consumed milk, however, was always found in the abomasum.

Lumps of curd were observed in the abomasum of every calf. There was considerable variation in the consistency of the lumps with different individuals. Though no specific measurements of hardness were made,

TABLE 2

Location of milk in stomach of calves slaughtered after consuming milk either from open pail or through nipple

Method of feeding	Number of calf	Breed	Sex	Age when slaughtered	Location of milk		
					Rumen and reticulum	Omasum	Abomasum
				Days			
Open pail	E–1	Holstein	Male	13	+	+	+
	E–2	Guernsey	Male	9	+	+	+
	E–3	Jersey	Male	10	–	+	+
	E–4	Jersey	Male	13	–	+	+
Nipple pail	E–5	Holstein	Male	11	–	+	+
	E–6	Holstein	Male	10	–	+	+
	E–7	Holstein	Male	13	–	+	+
	E–8	Guernsey	Male	11	–	+	+
	E–9	Guernsey	Male	13	–	+	+

+ = milk or curd present.
– = no milk or curd present.

from general examination the curd from the nipple fed calves appeared firmer and tougher.

The foregoing preliminary results relative to the channels followed by the milk ingested by different methods were substantiated from observations of calves having rumen fistulae. As indicated in Table 3, when the calves were fed milk from an open pail, a portion of the milk occasionally passed into the rumen and reticulum. The frequency of entrance ranged from zero in the case of 349–B to 78.8 per cent in the case of C–34. Considering the entire group, milk entered the rumen in 36.9 per cent of the cases. If

TABLE 3

Frequency of the entrance of milk into the rumen and reticulum and the amount entering when calves are fed either from open pail or through nipples

No. of calf	Open pail feeding					Nipple pail feeding		
	Total observations	Milk entered rumen				Total observations	Milk entered rumen	
		Observations		In amts.* > 25 mls.			Observations	
	No.	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*
C–29	186	36	19.4	3	8.3	24	0	0
C–30	73	40	54.8	16	40.0	26	4**	15.4
C–32	149	93	62.4	41	44.1	84	6**	7.1
C–34	118	92	78.0	59	64.1	75	0	0
C–36	125	68	54.4	22	32.4	79	0	0
C–37	78	20	25.6	2	10.0	104	0	0
349–B	216	0	0.0	0	0.0	65	0	0
Total	945	349	36.9	143	41.0	457	10**	2.2

* Amount of milk entering rumen was estimated.
** Small quantity, never more than 3 mls., during first swallows.

349-B, in which no milk entered the rumen, be excluded, the frequency of entrance is increased to 47.9 per cent.

The amount of milk entering as well as the frequency of entrance is important to the well-being of the calf. As shown in Table 3, in only 41 per cent of the cases in which the milk escaped into the rumen, the amount exceeded 25 mls. Though it was difficult to estimate the volume of milk in the rumen, the quantity rarely exceeded 50 per cent of the total ingested. The greatest proportion of the milk escaping into the rumen entered during the first few seconds of drinking.

When the milk was sucked through a valved nipple, the results were somewhat more consistent than when drunk from a pail. In only 2.2 per cent of the observed cases did any milk flow into the rumen. The amount entering never exceeded 3 mls., which spurted from the esophageal groove during the first several swallows.

The water, consumed by either of the two methods, entered the rumen more frequently than did milk ingested in a similar manner. As shown in Table 4, when water was drunk from an open pail, the frequency with which it passed into the rumen was high and relatively consistent with different individuals. In contradistinction when water was sucked through a nipple, the frequency of entry into the rumen was not only low but also variable.

TABLE 4

Frequency with which water entered the first compartments of the stomachs of calves when either drunk from pail or sucked through a nipple

No. of calf	Drinking from open pail			Sucking through nipple		
	Total observations	Water entered rumen		Total observations	Water entered rumen	
		Observations			Observations	
	No.	*No.*	*Per cent*	*No.*	*No.*	*Per cent*
C-29	45	38	84.4	8	0	0.0
C-30	16	16	100.0	8	3	37.5
C-32	78	77	98.7	16	15	93.7
C-34	86	82	95.3	16	4	25.0
C-36	80	79	98.7	30	10	33.3
C-37	33	32	97.0	22	1	4.5
349-B	77	75	97.4	27	5	18.5
Total	415	399	96.1	127	38	29.9

The amount of water escaping from the esophageal groove was proportionally great when taken by the pail method. The major portion of the water consumed usually flowed into the rumen. When the nipple method was used, only a relatively small proportion of the volume consumed entered the rumen.

DISCUSSION

It is obvious from the foregoing results that milk frequently escapes into the ruminal and reticular cavities when it is drunk from an open pail.

The time of entrance of the greatest amount was usually during the first few seconds of drinking. The detrimental effects of the presence of milk in these first two compartments of the stomach of the calf have been emphasized from nutritional and physiological standpoints. Schalk and Amadon (8) stated that milk undergoes digestive changes only within the abomasum; hence the passage of milk into the rumen and reticulum merely delays the assimilation of nutrients needed by the body. Akssenowa (1), however, assumed that enzymes of the abomasum of kids were also present and active in the rumen. This assumption was based on the observation of the transference of milk from the abomasum into the rumen. Though it is possible that similar transfers may take place in calves, this phenomenon was not observed in any of the foregoing experimental subjects. Mangold and Usuelli (7) reported that milk is deleterious to rumen infusoria. This may interfere with the normal functions, digestive or otherwise, of the rumen. Even if it is assumed that milk undergoes decomposition in the rumen, the extent to which the products may be absorbed directly remains a moot point. In accord with the observations of Schalk and Amadon (8) it was observed that the milk diluted with other fluids eventually passes from the ruminal cavity. Thus it seems that a consideration of the problem from any angle leads to the conclusion that the presence of large amounts of milk in the rumen and reticular cavities of calves probably retards digestion and assimilation.

Further indictment of pail-feeding milk is frequently made on the basis of digestive disturbances resulting from abnormal decomposition of the milk in the rumen. This, no doubt, is true when the major proportion of the ingested milk escapes into this cavity. The relative infrequency with which large quantities of milk entered the rumen suggests that this point has been overemphasized. In only one calf, C–34, did the milk frequently enter in prodigious amounts throughout the observation period. This was the only case in which the presence of milk in the rumen could be associated with diarrhea.

When the rumen wall was opened, considerable putrid curd was present in the cavity. Caseous necrosis was present on much of the area surrounding the esophageal groove. Nearly all the milk consumed from a pail gushed from the cardia directly into the rumen. Upon changing to a nipple, the necrotic condition gradually disappeared and scours subsided within several days. The crux of the problem is probably a matter *a priori*. Did the passage of the milk into the rumen initiate the pathological condition of the esophageal groove, or did some other factor cause the improper functioning of the groove permitting the passage of nearly all the milk into the rumen, thus aggravating the condition?

Apparently the older the calves the greater the frequency of entrance and the larger the volume of milk passing into the rumen; yet physiological disturbances rarely occur in older calves if they are receiving a normal ration of hay and grain. When calves are restricted to a whole milk ration over a prolonged period, curd frequently accumulates in the rumen (14). Gaseous putrefaction or other abnormal decomposition follows causing marked disturbances. Thus the nature of the ration fed in conjunction with the milk evidently is one factor determining the magnitude of the deleterious effects of milk in the rumen.

In view of the possible detrimental results of milk in the rumen, the obvious advantage of the nipple systems of feeding is the passage of milk directly from the cardia to the reticulo-omasal orifice without any significant spillage into the ruminal and reticular cavities. Furthermore, Espe (3) has suggested that the nipple system enhances complete coagulation in the abomasum as a consequence of slow ingestion and thorough mixing with saliva, which increases viscosity. This completeness of coagulation prevents flooding of the small intestine, which is rather sensitive. This, however, is not entirely in harmony with Sheehy's (9) recommendation that the milk be diluted with water in order to alter favorably the character of the rennin clot so as to facilitate rapid digestion. Any system that promotes quick coagulation with the formation of a soft curd would be considered ideal; yet this is contrary to *in vitro* observations (10). The extent to which consumption of milk through a nipple effects this desirable phenomenon in the abomasum of the calf is problematical.

The frequency with which water, when taken from an open pail, flows directly from the cardia into the rumen cavity is in accord with the report of Dukes (2). Evidently after the calf is several weeks of age the tonicity of the esophageal groove is not affected by water. Apparently nature has synchronized the time that this liquid begins to enter the rumen with the age at which the calf begins to consume roughage and concentrates in appreciable amounts.

When water is consumed via nipple, the relative infrequency of its passage into the rumen cavity and the small amount entering indicates that sucking produces a stimulus that causes the esophageal groove to function. Perhaps it is as abnormal for a calf to consume water by sucking as it is to ingest milk by drinking.

There is much variation in the responses of various calves and in the same individual at different times. The causes of these variations are numerous and indefinite. Some of the probable factors involved are being studied further.

SUMMARY

1. The frequency of the passage of milk and water into the ruminal and reticular cavities of calves was studied by administering the liquids from open pails and through valved nipples. Observations were made by slaughtering and by inspection through rumen fistulae.
2. There were marked variations in the responses of the calves.
3. When milk was ingested from an open pail, it frequently entered

the rumen in small amounts but rarely in quantities equivalent to the major portions consumed.

4. When milk was sucked through a valved nipple, it rarely entered the rumen cavity and never in significant amounts.

5. Water consumed from an open pail frequently flowed from the cardia into the rumen in amounts almost equal to the quantity consumed.

6. Water sucked through a nipple occasionally spurted into the rumen but in relatively small amounts.

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THE RELATION OF PHOSPHOLIPIDS TO FAT IN DAIRY PRODUCTS

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It is apparent from a study of the literature on the phospholipid content (as lecithin) of dairy products that there is a wide variation in the per cent "lecithin" between different milk products and also in the per cent "lecithin" in the fat extracted from the different products by the Mojonnier method.

This paper, in a measure substantiates previous work on the "lecithin" content of dairy products and offers an explanation for the results obtained.

REVIEW OF THE LITERATURE

Chapman (4) found that the per cent "lecithin" in the fatty extract obtained by the Mojonnier method was highest in buttermilk (20.25%), next highest in skim milk (10.78%) and milk (1.6%) and lowest in cream (.43%). This agrees with the work of Horrall (8) who reported as follows: buttermilk (19.66%), skim milk (13.91%), raw sweet cream (.428%), milk (.85%), butter from raw sweet cream (.232%). Holm, Wright, and Deysher (6) reported the following amounts as per cent lecithin in the product: whole milk, .0337; skim milk, .0169; 41% cream, .1816; butter, .1819; buttermilk, .1872. When calculated on the basis of per cent "lecithin" in the fat, these figures show close agreement with the results of Horrall and Chapman.

Perlman (13) showed that the phospholipid content of the fatty extract of fresh cream shows a variable decrease with the increase in the fat content of the cream indicating a variable loss of the original milk phospholipids in the skim. The lipide phosphorous per unit of fat was found to decrease between 58 and 80 per cent fat. Close agreement with the foregoing was reported by Jack and Dahle (10).

An extensive review of the literature on the "lecithin" content of dairy products is beyond the purpose of this paper and hence will not be presented; however, should one be desired, see Horrall (8) and Holm, *et al.* (5).

METHODS AND RESULTS

The term phospholipid is used to designate the fat like substances containing phosphorus that are extracted from milk and its products by the Mojonnier method. For convenience of comparison of the results obtained here with other results, the phospholipid was reported as per cent "lecithin" both in the product and in the fat extracted from the product using the for-

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mula given by Horrall (8), which closely approximates those of Bird, *et al.* (1), Holm, *et al.*, (6), and others.

Phospholipid was determined as described by Horrall (8) except that the fat was ashed carefully over a Fisher burner and that Nessler tubes were used instead of a colorimeter. Samples were run in duplicate; on the basis of per cent "lecithin" in the product, the difference between duplicate determinations varied from 0.000 per cent to 0.019 per cent and averaged 0.0046 per cent for all samples. Blank determinations were run frequently to insure freedom from contamination.

The milk and other dairy products used were as delivered to or made in the plant (with the exception of the cream and skim milk which were separated from tank milk in the laboratory) and all were of excellent quality. Milk was separated at 120° F. in a DeLaval S-16 separator. Creams over 60 per cent butterfat were obtained by separating 40 per cent cream. This procedure was found to give a product with the least amount of damaged fat globules. Skim milk from this operation is referred to as skim milk from reseparated cream. The butter and buttermilk were taken from commercial churnings.

All "lecithin" determinations were begun within two hours after the samples were obtained since it was found that the "lecithin" content of cream, skim milk and milk decreases rapidly with age, even when stored at 40° F. This observation is in agreement with the work of Cusick (3) Perlman, (14) and Rimpila and Palmer (15).

In experiments with washed creams, the volume of the original milk was measured (five gallons) and the resulting cream was diluted to this same volume with water at 100° F. in order to approximate the same fat percentage in the milk and maintain the same fat percentage in the cream.

"Lecithin" content of cream

The results of Perlman (13) and Jack and Dahle (10) show that as the fat content of cream increases to about 55 per cent there is a uniform increase in the phospholipid content. But above 60 per cent, they found that there was a decrease. Although they do not so state, it can be assumed that their creams were all separated from whole milk. In the results shown in Table 1 the creams over 60 per cent were separated from 35–40 per cent cream. This difference in method probably accounts for the difference in results since oiling off tests (11), microscopic examination, and general appearance of creams of high fat percentage obtained by reseparating 40 per cent cream indicated no churned or "free" fat.

Table 1 is composed of the results of several separation trials from several batches of milk extending from October to February. The milk to be separated was taken from a tank of about 10,000 pounds of milk after thorough mixing. The "lecithin" content of the milk was determined for each trial and varied from .035 per cent to .036 per cent.

TABLE 1

The "lecithin" content of cream of varying fat percentage

Fat (Mojonnier), per cent	"Lecithin" in product, per cent	"Lecithin" in extracted fat, per cent
14.14	.066	.463
16.84	.077	.459
18.89	.089	.469
21.54	.087	.406
21.90	.081	.367
22.44	.084	.374
32.00	.112	.349
36.23	.124	.344
38.45	137	.358
38.48	126	.327
38.98	.146	.374
39.10	.145	.372
39.51	.138	.349
40.23	.156	.387
40.87	.156	.382
41.00	.154	.376
41.29	.152	.369
41.78	.160	.383
41.81	.151	.359
43.69	.156	.358
43.98	.164	.374
48.11	.153	.318
50.63	.163	.323
53.34	.165	.309
54.30	.162	.300
55.81	.187	.335
56.89	.179	.314
68.43	.211	.308
72.26	.196	.271
75.21	.210	.279
75.79	.194	.256
76.15	.199	.261

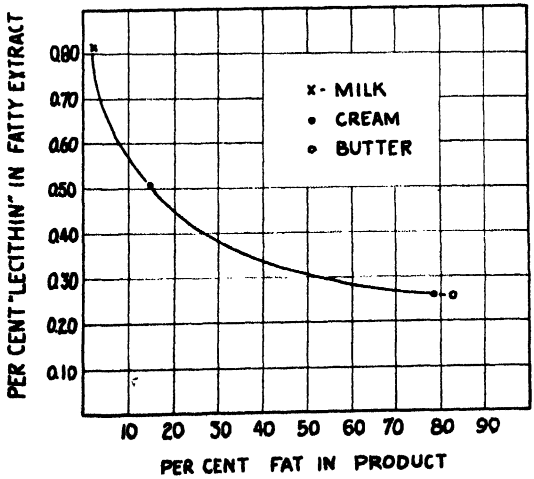

FIG. 1. The relation between the per cent "lecithin" in the fatty extract and the per cent fat in the product. (Buttermilk and skim milk not shown.)

Figure 1 shows the relation of the per cent "lecithin" in the cream (and other dairy products) to the per cent of fat in the cream, while Figure 2 represents the relation of the per cent "lecithin" in the fatty extract to the per cent fat in the cream (and other dairy products).

"Lecithin" content of other dairy products

The results shown in Table 2 for the "lecithin" content of other dairy products are in general agreement with the results of others. The per cent "lecithin" in the fatty extract is not constant but varies considerably among

TABLE 2

The "lecithin" content of dairy products

Product	Fat (Mojonnier), per cent	"Lecithin" in product, per cent	"Lecithin" in extracted fat, per cent
Skim milk	.075	.015	19.65
" "	.077	.018	24.05
" "	.066	.017	25.45
" "	.086	.017	19.79
" "	.080	.017	21.20
" "	.103	.017	16.55
" "	.077	.017	22.00
" "	.080	.016	19.97
" "	.089	.017	18.89
" "	.106	.016	14.78
" "	.096	.016	16.97
" "	.080	.018	21.90
" "	.090	.017	19.10
" "	.126	.018	14.52
" "	.076	.016	20.55
" "	.088	.016	18.42
Whole milk	5.01	.036	.720
" "	4.87	.035	.713
" "	5.50	.036	.655
" "	5.08	.036	.710
" "	5.10	.035	.691
" "	4.66	.035	.752
" "	4.71	.036	.765
" "	4.81	.035	.729
Sep. slime	1.12	.229	20.55
Buttermilk	.672	.114	16.93
"	.764	.128	16.81
"	.644	.126	19.55
Butter (sweet cream)	80.84	.216	.268
" " "	80.25	.209	.259
Butter (9 months in cold storage)	82.93	.165	.199
" " " " " "	82.69	.153	.184
Skim milk from 60–80% cream	.540	.093	17.25
" " " " "	.435	.084	19.36
" " " " "	.460	.080	17.40
" " " " "	1.378	.082	5.918
" " " " "	.196	.035	18.08
" " " " "	.192	.049	25.40

the various dairy products. It is apparently highest in skim milk, followed by buttermilk, milk, light cream, heavy cream, and butter.

The effect of washing on the "lecithin" content

The work of Rimpila and Palmer (15), Thurston, *et al.* (16), and Jack and Dahle (10) was repeated controlling as many factors as possible. Acidities were run to ascertain the amount of plasma present; oiling off tests (11) were run to determine the amount of free fat present after each washing. It was felt that as the oiling off increased, the amount of phospholipids originating from the fat might be increased, and this would give a false idea of the amount removed merely by washing. For this reason only 5 washings were employed.

Table 3 presents the decrease in the "lecithin" content of a cream containing 21.90 per cent fat and a cream containing 38.48 per cent fat. In addition is shown the observed decrease of "lecithin" due to washing and the calculated decrease.

TABLE 3

Effect of washing cream on the "lecithin" content

Sample		Fat, per cent	Acidity, per cent	Oiling off, units	"Lecithin" in product, per cent	Decrease in per cent "lecithin" due to washing	
						Observed	Calculated*
Trial I	Original	21.90	.125	.00	.081	.022	.020
	1st washing	19.46	.040	.02	.062		
	3rd "	21.49	.020	.06	.059		
	5th "	21.79	.015	.14	.059		
Trial II	Original	38.48	.105	.01	.126	.020	.021
	1st washing	36.19	.030	.02	.112		
	3rd "	40.93	.020	.17	.106		
	5th "	44.26	.015	.30	.107		

* Calculated by multiplying the per cent plasma in the product by the per cent "lecithin" in the plasma.

DISCUSSION

Altho Horrall (8) states that possibly "the lecithin is a separate constituent of milk and is not tied up with the fat," Bird, *et al.* (2), North and Sommer (12) and Jack and Dahle (9) conclude from their work that the phospholipid material is closely associated with the fat globule. Thurston, *et al.* (16) have shown that "agitation of milk caused a reduction in the lecithin content of the cream and a corresponding increase in the lecithin content of the skim milk." That this reaction is not reversible is shown by the work of Rimpila and Palmer (15) who studied the membrane of pure fat globules dispersed in skim milk and found that the normal fat globule membrane is not derived from milk plasma.

Nevertheless, the results presented here indicate that there are some phospholipids in the plasma of milk. Since the method of determining "lecithin" depends upon the determination of ether-alcohol soluble phosphorous, the method may give erroneous results. Indeed, Holwerda (7) states that there is a phosphoric ester found in milk which gives too high a value for phospholipid percentage. This indicates that possibly the plasma contains a substance extracted by the alcohol and ethers which contains phosphorus and which would vary with the amount of plasma in the dairy products. This substance, however, is not inorganic phosphates since Bird, *et al.* (1) and Wiese, Nair, and Fleming (17) state that these are not extracted. Trials have been made here which also substantiate this conclusion.

It seems, therefore, that the material extracted is organic in nature, yet that some of it is present in the plasma of milk. If simultaneous equations be set up as follows:

$$\left(\frac{12.2}{100}\right) x + \left(\frac{87.8}{100}\right) y = .060$$

$$\left(\frac{15.5}{100}\right) x + \left(\frac{84.5}{100}\right) y = .070$$

In which x = the per cent "lecithin" in the fat and y = the per cent "lecithin" in the plasma. The per cent "lecithin" in a cream with 12.2 per cent fat and 87.8 per cent plasma is .060; .070 is the per cent "lecithin" in a cream with 15.5 per cent fat and 84.5 per cent plasma. (This data was obtained from Figure 2.) Solving, x = 0.326% and y = 0.0230%.

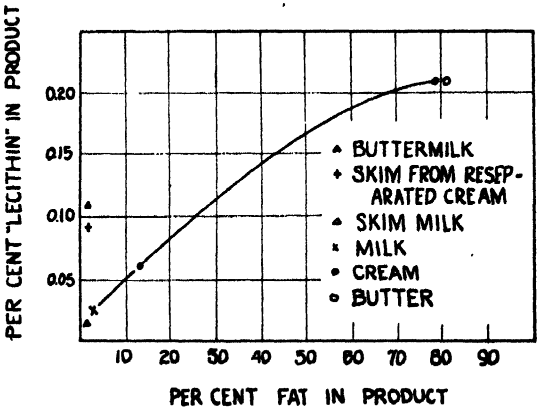

FIG. 2. The relation between the per cent "lecithin" in the product and the per cent fat in the product.

By solving many such equations and taking an average of the various x and y values, results are obtained which approximate the per cent phospholipid in the plasma and in the fat of creams of various fat percentages.

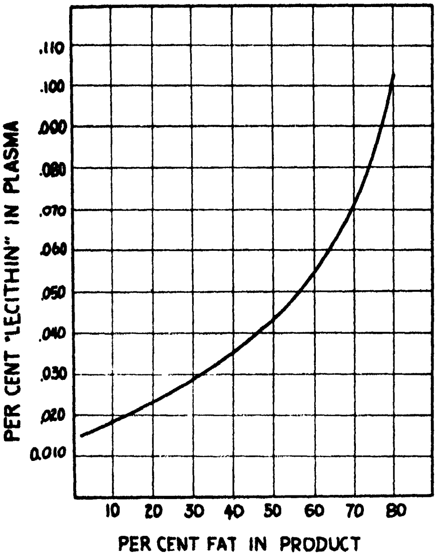

FIG. 3. The relation between the per cent "lecithin" in the plasma and the per cent fat in the product. (Not to be applied to buttermilk and skim milk from reseparated cream.)

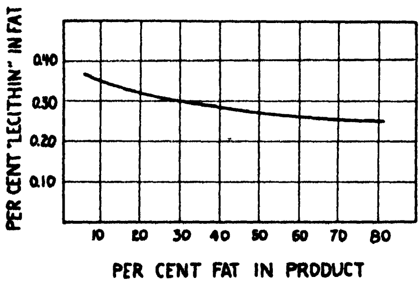

FIG. 4. The relation between the per cent "lecithin" in the fat and the per cent fat in the product.

These values are shown graphically in Figures 3 and 4 which may be further explained as follows:

In the milk products considered here a portion of the "lecithin" is associated with the fat and a portion is in the plasma phase. Figures 3 and 4 give the percentages of lecithin in the plasma and associated with the fat, respectively, as a function of the fat content of the product. The total "lecithin" content of the product can be calculated by dividing the percenage of fat in the product by 100 and multiplying by the corresponding percentage of "lecithin" (Figure 4) and adding to this value the result obtained by dividing the percentage of plasma in the product by 100 and multiplying by the corresponding percentage of "lecithin" in the plasma

(Figure 3). For example, the percentage of "lecithin" in 40 per cent cream would be calculated as follows:

$$\left(\frac{40}{100}\right)(0.29) + \left(\frac{60}{100}\right)(.035) = 0.137\%$$

(It must be pointed out that these figures may be found to apply only to milk from this section of the country and produced during the winter months.)

If the above supposition is true, then by washing cream of a given fat percentage one should be able to calculate the decrease in the per cent "lecithin" in the plasma from the total amount. This result is recorded in Table 3 and the calculated and observed decreases check closely.

The high "lecithin" content of buttermilk and skim milk from reseparated cream, may be explained by this method. According to Figure 3, the plasma of 75 per cent cream contains 0.09 per cent "lecithin." This plasma has the same composition as the skim milk resulting during the separation of 75 per cent cream. Consequently, it would have .09 per cent "lecithin" in its plasma. Since the plasma in such skim milk is about 99.9 per cent, there would be:

$$\left(\frac{99.9}{100}\right) .09 + \left(\frac{.1}{100}\right) .25 = .090\%$$

This agrees with the measured amount present. The "lecithin" content of buttermilk may be explained on the same basis.

It is possible that the statement of Thurston, *et al.* (16), previously referred to explains the increase in "lecithin" in the plasma as the fat in the product is increased, *i.e.*, there is a greater movement of "lecithin" from the fat to the plasma due to the increased mechanical agitation during the separation of creams of higher fat percentage.

SUMMARY AND CONCLUSIONS

1. The per cent "lecithin" in various dairy products was found to be as follows: skim milk, 0.015–0.018 per cent; raw whole milk, 0.035–0.036 per cent; skim milk from reseparated cream, 0.035–0.093 per cent; pasteurized sweet cream, 0.066–0.199 per cent; buttermilk, 0.114–0.126 per cent; butter, 0.153–0.212 per cent; separator slime, 0.229 per cent.

2. The "lecithin" in the products studied was found to be present partially in the plasma and partially associated with the fat.

3. The results obtained indicate that as the fat content of the product increases, there is an increase in the relative amount of "lecithin" in the plasma and a decrease in the relative amount associated with the fat, except in buttermilk and skim milk from reseparated cream.

4. The "lecithin" content of the fat and plasma in buttermilk and skim milk from reseparated cream is approximately the same as the "lecithin" content in the fat and plasma of the butter and 75–80 per cent cream from which they are derived. This accounts for the abnormally high "lecithin"

content of these two products compared to other products of a like fat content, *i.e.*, skim milk and whole milk.

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SOME FACTORS AFFECTING THE STABILITY OF CERTAIN MILK PROPERTIES. I. EFFECT OF SUCCULENT ROUGHAGES ON FLAVOR*

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Anderson (1, 2) seems to have been the first worker to report that there was an apparent relationship between carotene in the feed of the cow and the ability of the cow to produce a milk of good flavor. His work was followed by experiments (3, 4) at the New Jersey Agricultural Experiment Station in 1937 and reported in 1938 in which it was shown that there was a high positive correlation between yellow color and good flavor in milk. Hening and Dahlberg (5) however, concluded "that the feeding of mangels or beet pulp in no way prevented or increased the susceptibility of milk to the development of oxidized flavor." Beet pulp and mangels are low in carotene.

To study more completely the stability of flavor in milk, experiments were carried out during the winter months of 1937–1938 in which 4 purebred Holstein and 8 purebred Guernsey cows, carefully selected as to stage of lactation, were fed alternately on roughage rations of beet pulp, corn silage and molasses grass silage. Earlier work had indicated that beet pulp tends to deplete yellow color in milk even when fed for a comparatively short time (2–3 weeks) so on this basis it was selected as the control or basic roughage ration. The cows were matched as nearly as possible as to the color of their milk and were divided into two equal groups. The roughage feeding program was as follows for the two groups:

	Group I	*Group II*
Three weeks	Beet pulp	Beet pulp
One week	Transition	Transition
Three weeks	Grass silage	Corn silage
One week	Transition	Transition
Three weeks	Beet pulp	Beet pulp
One week	Transition	Transition
Three weeks	Corn silage	Grass silage
One week	Transition	Transition
Three weeks	Beet pulp	Beet pulp

In addition the cows received the usual grain ration and a rather poor quality field-cured hay throughout the experiment.

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TABLE 1

Average flavor scores

	Beet pulp		Grass silage		Beet pulp		Corn silage		Beet pulp	
	– Cu*	+ Cu**	– Cu	+ Cu	– Cu	+ Cu	– Cu	+ Cu	– Cu	+ Cu
					Group I					
Raw milk										
First day	20.63	20.42	22.33	21.42	21.03	20.47	20.93	20.54	20.78	19.78
Third day	19.98	18.65	21.63	20.63	21.04	19.67	20.25	19.38	19.00	17.92
Fifth day	17.56	15.44	20.56	19.11	17.50	15.69	17.06	16.48	17.56	14.63
Pasteurized milk										
Second day	20.81	20.03	22.47	21.11	21.29	20.29	20.84	19.56	20.43	17.49
Fifth day	18.86	16.02	20.71	18.33	19.58	14.61	18.65	14.73	17.70	14.36
					Group II					
	Beet pulp		Corn silage		Beet pulp		Grass silage		Beet pulp	
Raw milk										
First day	21.17	20.58	20.75	20.63	21.00	20.50	22.23	21.26	20.81	20.11
Third day	19.17	18.50	20.72	19.44	20.38	19.50	21.57	20.48	19.36	17.59
Fifth day	16.86	15.86	18.00	16.97	17.39	15.17	18.90	18.02	17.22	15.33
Pasteurized milk										
Second day	21.39	19.67	20.83	19.72	21.21	20.29	22.11	20.75	20.64	18.14
Fifth day	17.34	15.77	18.92	16.04	19.28	15.11	20.11	17.27	18.45	14.20

* – Cu—No copper.

** + Cu—Contains 1.3 parts copper per million parts milk.

Samples of milk from each cow were taken once each week for analysis. Half of each sample was stored raw and half was pasteurized before storage. To half of each of the raw and the pasteurized milks was added copper sulfate in amounts to give a concentration of 1.3 parts copper per million parts of milk. The raw milks were checked for flavor score and intensity of oxidized flavor on the first, third and fifth days. The pasteurized samples were checked on the second and fifth days.

In Table 1 are shown the average flavor scores of the milks for the two groups produced on the three different roughages. Only the scores of the milks obtained at the end of each roughage feeding period are used in arriving at the averages. It was felt that these values are more truly representative of each feed than if the scores throughout each feeding period (three weeks) had been used since there is an apparent carry-over effect from previous feeds for several days. From the data obtained it is even doubtful that three weeks is sufficient time to reach maximum or minimum effects from the different feeds.

The data presented in Table 1 are summarized in Table 2. They definitely show that the milks produced on molasses grass silage were of better initial flavor, held their good flavor in storage better, and withstood the destructive effects of soluble copper better than those milks produced on either corn silage or beet pulp. This was not only indicated by the average score but is supported by the fact that the milk of every cow without exception showed improvement in flavor over either corn silage or beet pulp when the roughage ration was mainly grass silage. Not much difference in flavor quality could be noticed between milks produced on corn silage and those on beet pulp. The data, however, indicate a slight advantage for corn silage over beet pulp in the way the milks kept in storage or withstood copper contamination.

TABLE 2

Summary of average flavor scores

	Beet pulp		Corn silage		Grass silage	
	– Cu	+ Cu	– Cu	+ Cu	– Cu	+ Cu
Raw milk						
First day	20.90	20.31	20.84	20.59	22.28	21.34
Third day	19.82	18.64	20.49	19.41	21.60	20.56
Fifth day	17.35	15.35	17.53	16.73	19.73	18.57
Pasteurized milk						
Second day	20.96	19.32	20.84	19.64	22.29	20.93
Firth day	18.54	15.01	18.79	15.39	20.41	17.80

The color of the milks was measured by the "lactochrometer," an instrument developed by the New Jersey Station for the American Guernsey Cattle Club. The color values, expressed as "lactochrometer units," for the fresh raw milks analyzed on the day the samples were obtained from the cows are presented in Table 3 and summarized in Table 4. Again only the samples obtained at the end of each respective roughage feeding period were used for obtaining these values.

TABLE 3

Average color values

Group I				
Beet pulp 4.28	Grass silage 5.09	Beet pulp 4.43	Corn silage 4.59	Beet pulp 4.03
Group II				
Beet pulp 4.48	Corn silage 4.54	Beet pulp 4.01	Grass silage 5.43	Beet pulp 4.08

TABLE 4

Summary of average color values

Roughage	Color value
Beet pulp	4.22
Corn silage	4.57
Grass silage	5.26

The relation between high yellow color and good flavor, as found in the 1937 studies, is completely substantiated by these studies as shown by the data presented in Table 5 and the correlation coefficients listed in Table 6.

TABLE 5

Ranking of color and flavor by individual cows

Cow No.	Breed	Color (Fresh raw milk)		Flavor (Fresh raw milk)	
		Units	Rank	Score	Rank
465	Guernsey	5.89	1	21.47	2
247	"	5.46	2	21.422	3
164	"	5.36	3	21.51	1
422	"	5.33	4	21.34	6
179	"	5.07	5	21.35	5
475	"	5.06	6	20.92	10
471	"	5.05	7	21.419	4
431	"	4.81	8	21.14	7
92	Holstein	4.11	9	21.086	9
129	"	3.72	10	20.71	11
226	"	2.90	11	21.087	8
284	"	2.23	12	20.62	12

The rankings of the cows for flavor (Table 5) agree fairly well with those of color with the exception of No. 475. This cow had mastitis during the latter part of the experiment which brought her average flavor score down below what might have been expected under more favorable circumstances. Cow No. 422 also produced a salty milk toward the end of the experiment which lowered the flavor score somewhat.

More significant, perhaps, than the data presented in Table 5 are the correlation coefficients (product moment method) shown in Table 6. Coeffi-

TABLE 6

Correlation coefficients; color vs. flavor

	Samples at end of period		All samples	
	r	S.D.*	r	S.D.*
Raw milk without copper				
Color vs. flavor (1st day)	+ .5287 ± .0627**	0.7180 ± .0442**	+ .4691 ± .0359**	0.6833 ± .0222**
" " " (3rd ")	+ .5690 ± .0589	1.0214 ± .0720	+ .4928 ± .0348	1.2019 ± .0391
" " " (5th ")	+ .7747 ± .0348	2.2013 ± .1355	+ .5253 ± .0333	1.8693 ± .0608
Raw milk with copper				
Color vs. flavor (1st day)	+ .3848 ± .0742	0.6733 ± .0415	+ .3806 ± .0393	0.6225 ± .0202
" " " (3rd ")	+ .6286 ± .0583	1.5880 ± .0978	+ .5661 ± .0313	1.5003 ± .0488
" " " (5th ")	+ .6795 ± .0469	1.9857 ± .1223	+ .5634 ± .0314	1.7530 ± .0570
Pasteurized milk without copper				
Color vs. flavor (2nd day)	+ .5217 ± .0634	0.7772 ± .0479	+ .5294 ± .0332	0.7729 ± .0252
" " " (5th ")	+ .6600 ± .0491	1.4331 ± .0882	+ .5865 ± .0302	1.3040 ± .0425
Pasteurized milk with copper				
Color vs. flavor (2nd day)	+ .4153 ± .0721	1.4383 ± .0886	+ .3735 ± .0398	1.1514 ± .0375
" " " (5th ")	+ .5713 ± .0586	1.8601 ± .1145	+ .5392 ± .0327	1.6349 ± .0533

Standard deviation of color (samples at end of period) = 1.1692 ± .0720.
Standard deviation of color (all samples) = 1.1261 ± .0367.
* Standard deviation of flavor.
** Probable error.

TABLE 7

Average loss of flavor

	Without copper				With copper			
	Third day*		Fifth day		Third day*		Fifth day	
	Points	Per cent	Points	Per cent	Points	Per cent	Points	Per cent
				Raw milk				
All samples	1.02	4.87	2.91	13.66	2.04	9.64	4.33	20.49
Guernsey samples	0.82	3.88	2.41	11.09	1.80	8.45	4.06	19.10
Holstein samples	1.42	6.84	3.91	18.81	2.51	12.02	4.85	23.28
Beet pulp samples	0.99	4.72	2.91	13.90	2.30	10.95	4.83	23.01
Corn silage samples	0.85	4.10	3.00	14.44	1.61	7.75	3.85	18.45
Grass silage samples	1.14	5.20	2.80	12.75	1.92	8.74	3.75	17.04
				Pasteurized milk				
All samples	+0.02	+0.10	2.16	10.22	1.15	5.41	5.21	24.51
Guernsey samples	+0.04	+0.22	1.89	8.87	1.18	5.51	5.05	23.55
Holstein samples	0.02	0.12	2.70	12.93	1.10	5.23	5.53	26.45
Beet pulp samples	+0.03	+0.18	2.20	10.47	1.14	5.35	5.40	25.76
Corn silage samples	0.06	0.31	2.25	10.78	0.96	4.61	5.02	23.92
Grass silage samples	0.06	0.25	2.16	9.83	1.14	5.18	4.65	21.15

* Second day for pasteurized milk.

cients were calculated on the basis of only those samples taken at the end of each roughage feeding period and also on the basis of all samples taken during the experiment.

It will be observed that the significance of the correlation between color and flavor increases as the storage period increases. This is true of both raw and pasteurized milks and also of those samples containing copper. It will be observed also that the correlation is relatively insignificant when copper is first added to the milk but that the significance greatly increases as the milk ages. This probably is because the metallic taste at first imparted to the milk by the copper tends to bring the flavor of all samples to a fairly common level. Later on with the onset of oxidation and the development of oxidized flavors the correlation becomes more significant.

In Table 7 are shown the average losses of flavor of raw and pasteurized milks without and with added copper. The per cent of loss of each case is calculated on the basis of the score of the raw fresh milk as 100 per cent. The slight gains in flavor for second-day pasteurized milk over first-day raw milk are due to improvement in flavor by pasteurization which frequently happened when the raw milk exhibited a feedy flavor. Both raw and pasteurized milks produced on grass silage showed an actual as well as a percentage loss less than those milks produced on either beet pulp or corn silage.

The high negative correlation coefficients presented in Table 8 definitely show that milks of high yellow color tend to lose less of their flavor in storage than do milks of lower color. This seems to indicate that carotene, or some factor associated with carotene, when present in sufficient quantity has a

TABLE 8

Correlation coefficients, color vs. loss of flavor

	r	S.D.*
Raw milk		
Without copper		
Color vs. loss of flavor (third day)	−.8902 ± .0404**	1.6988 ± .2339**
Color vs. loss of flavor (fifth day)	−.9062 ± .0348	3.8435 ± .5292
With copper		
Color vs. loss of flavor (third day)	−.8176 ± .0645	2.7953 ± .3849
Color vs. loss of flavor (fifth day)	−.7659 ± .0805	3.4747 ± .4784
Pasteurized milk		
Without copper		
Color vs. loss of flavor (second day)	−.6395 ± .1151	0.7651 ± .1053
Color vs. loss of flavor (fifth day)	−.9115 ± .0329	2.2418 ± .3087
With copper		
Color vs. loss of flavor (second day)	−.3108 ± .1759	1.6834 ± .2318
Color vs. loss of flavor (fifth day)	−.5464 ± .1366	3.5631 ± .4906

Standard deviation of color = 1.0689 ± .1472.

* Standard deviation of flavor score.

** Probable error.

stabilizing effect on milk flavor. The low coefficient of second-day pasteurized milk with copper is probably due to the fact that the copper has tended to bring all the milks to a fairly common flavor level, as explained above, but has not had time to produce a high degree of oxidized flavor.

The degree or intensity of oxidized flavor in a milk induced by the addition of soluble copper should be a fairly satisfactory index of the efficiency of a feed in preventing or retarding oxidation if our theory that certain feeds contain inhibitors or antioxidants is correct. The data in Table 9 do show a wide variation in the intensity of the flavor when milk is produced on roughages of different carotene content. The oxidized flavor scores presented in this table are obtained by assigning the numbers, 0, 1, 2, 3, 4, 5, to represent the different degrees of oxidation. A number 1 represents a barely perceptible oxidized flavor while a number 5 indicates a milk of such high intensity of flavor that it is practically unsalable. Here again molasses grass silage appears quite superior to either corn silage or beet pulp for producing a milk which will resist the development of oxidized flavors. Although not shown in the data presented in this paper there was a considerable number of cases where, without the addition of copper, oxidized flavor appeared in the beet pulp or corn silage milk, but not in a single instance did this occur in the grass silage milk. In fact where oxidized flavor appeared previous to the feeding of grass silage, the condition cleared up within a short time after grass silage feeding was begun.

TABLE 9

*Distribution and intensity of oxidized flavor**

	Raw milk		Pasteurized milk	
	Third day	Fifth day	Second day	Fifth day
Samples at end of period				
All samples	0.91	2.25	0.73	3.08
Beet pulp samples	1.25	2.86	0.95	3.39
Corn silage samples	0.67	1.58	0.58	3.42
Grass silage samples	0.17	0.92	0.25	1.83
All samples				
All samples	0.98	2.03	0.63	2.88
Beet pulp samples	1.30	2.71	0.79	3.16
Corn silage samples	0.67	1.47	0.47	2.86
Grass silage samples	0.47	1.00	0.28	2.03

* All samples contained added copper.

An analysis of the hydrolytic rancid flavor which developed in the raw milks used in this study is presented in Table 10. The same numerical values were used for the intensities of the rancid flavor as were employed for the oxidized flavor. These data do not seem quite so significant as in the case of the oxidized flavor, especially if the data on all samples throughout each feeding period are taken. But if only the samples at the end of each feeding period are used, then it appears that the feeding of grass silage had some effect in preventing the development of an intense rancid flavor. The lack of significance in the former case and the occurrence of significance in the latter suggests that a longer feeding period on grass silage might help to greatly inhibit the development of rancid flavor.

TABLE 10

Distribution and intensity of rancid flavor

	Samples at end of period		All samples	
	Third day	Fifth day	Third day	Fifth day
All samples	0.90	1.83	0.91	1.59
Beet pulp samples	0.95	1.94	0.91	1.59
Corn silage samples	0.92	2.00	0.97	1.94
Grass silage samples	0.42	1.33	0.81	1.53

When the original scores of the fresh raw milks were correlated against the corresponding per cents loss of flavor during storage, high negative correlation coefficients were obtained. This indicates that milks of high original flavor score will retain their flavor in storage better than milks with beginning low flavor scores. The correlation coefficients are shown in Table 11.

TABLE 11

Correlation coefficients; original flavor score vs. percentage loss

	r	S.D.*
Raw milk without copper		
Third day	−.8300 ± .0606**	1.6988 ± .2339**
Fifth day	−.7577 ± .0829	3.8435 ± .5292
Raw milk with copper		
Third day	−.7981 ± .0707	2.7953 ± .3849
Fifth day	−.8317 ± .0600	3.4747 ± .4784
Pasteurized milk without copper		
Second day	−.6762 ± .1057	0.7651 ± .1053
Fifth day	−.8352 ± .0589	2.2418 ± .3087
Pasteurized milk with copper		
Second day	−.3641 ± .1689	1.6834 ± .2318
Fifth day	−.6202 ± .1198	3.5631 ± .4906

Standard deviation of original flavor score = 0.2862 ± .0394.

* S.D. = standard deviation of loss of flavor score.

** Probable error.

The results of this study tend to show on the whole that the feeding of molasses grass silage helps to stabilize the flavor of milk thus produced. This seems to be especially true with regard to the development of oxidized flavors. Molasses grass silage is considerably higher in carotene than is corn silage or beet pulp as shown by chemical analysis and evidenced by the greater amount of yellow color in the milk and the large amount of yellow secretion in the skin of the cows. The yellow skin secretion was especially noticeable in the

Holsteins which during the winter months, usually are almost entirely lacking in such secretion.

Shrewsbury and Kraybill (6) have shown that some substance associated with carotene in butterfat exhibits antioxidative properties toward the oxidation of the carotene. In studies on the keeping quality of butter (7) observations were made that the end of the induction period of accelerated oxidation of pure butterfat was invariably accompanied by rapid and almost complete bleaching of the yellow color. Immediately thereafter rapid absorption of oxygen by the butterfat began. This phenomenon may either mean that the carotene itself was acting as antioxidant toward the fat and that oxidation of the fat could not begin until all the carotene was oxidized or it may mean that some other unidentified natural antioxidant is normally present and the bleaching of the carotene is merely evidence that the activity of this natural antioxidant has been overcome and is the first step in the progressive oxidation of the butterfat proper.

Although the results of this study do not definitely prove that the carotene in the feed or in the milk is the true causative antioxidant factor, they do indicate definitely that it is an associative one. If the stabilizing influence comes into play only after the milk is drawn from the cow, it is possible that the substance postulated by Shrewsbury and Kraybill is the true causative factor. If, on the other hand, the stabilizing influence begins in the milk as it is being synthesized in the mammary tissue, as seems quite likely, then the carotene (or vitamin A) may be the true factor. This would be especially true if the carotene (or vitamin A) served to keep the mammary tissue in a healthy condition and functioning properly.

SUMMARY

The results of these studies indicate that:

The feeding of molasses grass silage to milking cows has the advantage over either beet pulp or corn silage in that it produces milk which:

(a) Has a superior initial flavor.
(b) Has a more stable flavor in storage.
(c) Is more stable toward the catalytic effects of added soluble copper.

The feeding of grass silage prevents the development of oxidized flavor in milk when it is not contaminated with soluble metal and greatly retards the development and lessens the intensity of the flavor when soluble copper is added.

Accelerating the oxidation reaction by adding soluble copper to milk seems to be a satisfactory means of demonstrating, more or less quantitatively, the relative stability of flavor of different milks.

The feeding of molasses grass silage seems to retard the development of hydrolytic rancidity in milk. The results presented in this article are not as conclusive as those on oxidation and the subject needs further study.

There is a significant positive correlation between the yellow color and the flavor of freshly drawn milk from individual cows.

A very significant negative correlation exists between the yellow color of the fresh milk and the loss of flavor in this milk during storage.

Milks of extra good flavor when fresh retain their good flavor in storage better than corresponding milks of poorer flavor quality.

The stabilizing effect on flavor of grass silage seems to be due, in part at least, to the comparatively high carotene content of this type of feed.

The feeding of properly ensiled green grass crops appears to be a highly satisfactory method of producing a milk of high flavor stability during winter months in northern climes.

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RAPID PHOTOELECTRIC DETERMINATION OF CAROTENOID IN MILK FAT

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INTRODUCTION

The importance of carotene as a precursor of vitamin A and as a yellow pigment has led to many studies in recent years on the carotene content of milk fat. There is need for a reliable method for determining carotene which is rapid enough so that a large number of samples can be studied and which does not require extremely elaborate and expensive equipment. We have devised a method by which the carotene or more precisely total carotenoid content can be determined directly in a sample of melted milk fat with the use of a photoelectric cell and suitable glass filters. No weighings or dilutions are necessary and only a few seconds are required for a determination after the melted fat has been prepared from cream. Three cubic centimeters of milk fat are needed for an analysis.

Several authors (1, 5, 8, 9) have encountered difficulties in the use of a simple colorimetric method for determining carotenoid concentrations. For example, the fields in the colorimeter are difficult to match since the color varies with concentration, temperature, and with the solvent. Also when read visually against dichromate, the depth of color is not proportional to concentration, and is three times as intense when carotene is dissolved in melted butter as when dissolved in petroleum ether. Some of these difficulties are avoided when a photoelectric colorimeter is used. Spectrophotometers of many modifications have been employed (1, 2, 3, 4, 8, 9). These instruments are expensive and are not available in most laboratories where problems on the color of milk fat are being studied.

APPARATUS

Design of photoelectric colorimeter

The photoelectric colorimeter we have used can be constructed very simply. A 30 candlepower automobile headlight bulb is used as a source of light in connection with a storage battery. It is important *not* to plug this light into a socket, but to solder wires directly to the base. This prevents light fluctuations due to heating of the contacts. Between the lamp and the absorption cells are placed two condensing lenses and an iris diaphragm by means of which the intensity of the light is controlled. A diaphragm from a microscope condenser is satisfactory for this use. The diaphragm is placed in a region of parallel light rays. The condensers are biologist's magnifiers

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which are sold in most student supply houses. They have a focal length of about one inch. They are so adjusted that the filament is at the focal point of one and the center of the absorption cell at the focal point of the other. The glass filters and the diaphragm are placed between the lenses. Adjacent to the absorption cell and between the absorption cell and the light is a slit about 4 mm. wide to cut out stray light. Behind the absorption cell is the photoelectric cell of the blocking layer type, which is connected to a microammeter with a full scale of 15 microamperes and an internal resistance of 150 ohms. Molded Corning glass filters two inches square were used. For carotenoid in milk fat, Nos. 585 and 428 were found to be the most suitable. Figure 1 is a diagrammatic sketch of the apparatus.

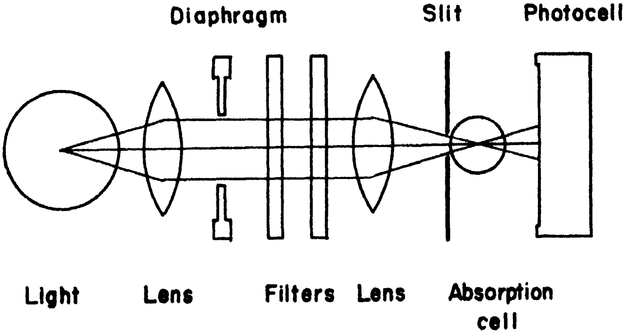

FIG. 1. Optical system of colorimeter.

The chief feature of our instrument which makes it convenient for work with milk fat is the type of absorption cells used. These cells are small test tubes, 10 cm. long, made from precision glass tubing with an inner diameter of 10.00 mm. ± .01 mm.* The tubing is not perfectly clear, but it is uniform. The imperfections have no significant effect on the microammeter readings, as can be readily demonstrated by rotating the tubes while obtaining the readings. The tubes have many advantages over the ordinary plane absorption cells with respect to ease of cleaning, cost of replacement, small quantities of liquid required, and rapidity of determinations. Grease never creeps on to the outside surface and the tubes can be tightly stoppered for use with volatile liquids. By focusing on the center of the cylindrical tubes more intense light is obtained than in the case of the plane cells which intercept parallel light. Ordinary test tubes can be used, but their diameters must be measured and for each determination the absorption coefficient must be calculated and corrected to 1.0 cm. With the precision tubes a single reading is recorded and the concentration of unknown obtained simply by reference to a table with no calculation being required.

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* This tubing, K. P. G., Duran glass, can be obtained from the Fish-Schurmann Corporation, New York City.

Calibration of the photoelectric colorimeter

The light absorption of pure carotene was determined in the apparatus by testing three samples of carotene dissolved in petroleum ether. One sample from Hoffmann La Roche was labeled 75 per cent beta carotene and 25 per cent alpha. Another from the S.M.A. Corporation was labeled carotene, and a third from the S.M.A. Corporation was labeled beta carotene. All these samples showed essentially the same light absorption (maximum deviation ± 5.3 per cent from the mean). The sample yielding the highest of these three values was assumed to be the purest because any likely impurities or decomposition would result in weaker light absorption.

Change in solvent produces a marked change in the color of carotene solutions. Thus the color in carbon bisulfide is much redder but the absorption is less intense than in petroleum ether. The filter combination selected for routine determinations of carotenoid in butter was the most insensitive to change in solvent or temperature.

Ordinarily those filters are selected for colorimetric work whose transmission band corresponds most closely to the maximum in the absorption band of the solution. The filter combination, yielding the highest value of absorption coefficient, would be most nearly ideal, because it would permit determinations in the most dilute solutions. However, in the case of carotenoid in milk fat it is advantageous to use a filter combination which yields a lower value for absorption coefficient. This is due to the high concentration of carotenoid in some samples which absorbs the light so nearly completely with "ideal" filters that accurate readings can not be obtained. With a less sensitive filter combination (such as 585 and 428) all the milk fat samples encountered by us absorbed the light incompletely and thus could be measured directly without dilution.

Another requirement for a filter combination is that the absorption coefficient of the substance to be tested should increase rapidly with concentration over the whole range. It is not necessary that there be direct proportionality with concentration. In fact direct proportionality is difficult to obtain without the use of monochromatic light. As a matter of fact deflections of the microammeter are not exactly proportional to light intensity unless a correction is made for the fixed resistance of the microammeter and the variable internal resistance of the photronic cell. By using a microammeter of low resistance this latter effect can be made very small. A method has been devised for eliminating this deviation (10) but simplicity is sacrificed. It was considered simplest to plot an empirical curve for a standard carotene solution with the actual readings depending on the characteristics of the filters, photocell, microammeter and light. The absorption coefficients reported here are more precisely the logarithms of the ratio of two microammeter readings rather than of two light intensities.

A calibration curve for the change in absorption coefficient with carotene concentration in cotton seed oil has been determined and is shown in Figure 2. Cotton seed oil was used as a solvent for the calibration curve instead of using butter itself as a solvent for the reason that corrections could be accurately made for the light absorption of the solvent alone. Small values of absorption coefficient for the solvent can be subtracted directly from the values for a solution containing added carotene to obtain the absorption coefficient of carotene. But this correction cannot be made by simple subtraction in solutions of high absorption as in the case of butter where there is no longer a linear relation between absorption and concentration.

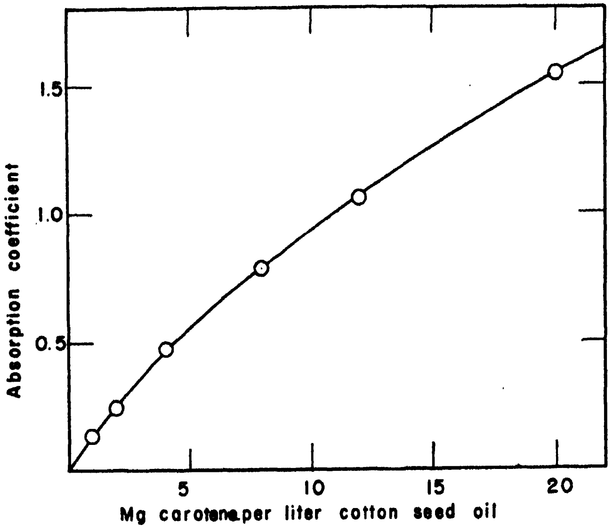

FIG. 2. Light absorption by carotene.

After the calibration curve is once obtained, none of the parts of the instrument can be changed without recalibration. It is particularly important to note that the glass filters and the photocells can not be exactly duplicated. For this reason the calibration curve shown in Figure 2 is correct only for the particular instrument in which it was determined. Moreover the positions of the light and photocell in the instrument must not be changed because the same region of the photocell surface must be illuminated in all determinations. It is very convenient to check the adjustment of the instrument each time that it is used by testing a standard solution of potassium bichromate containing 0.2024 grams c.p. potassium chromate in 1 liter of N/10 sulfuric acid. In our instrument this solution absorbs half the light* thus having an absorption coefficient of 0.301 with filters 585 and 428.

* We are indebted to Dr. Hugo Doob for the preparation of this solution and tests on its stability as well as for the observation that azobenzene could not be used because of its light sensitivity.

As a further rough check on the purity of the carotene preparations, the apparatus was calibrated with azobenzene. It was found that a solution of 14.5 mg. azobenzene in 100 cc. of 95 per cent alcohol corresponded to a solution of 2.28 mg. carotene per liter. This value is in agreement with the 2.35 mg. found by Kuhn and Brockmann (5) for both alpha and beta carotene, using a cobalt glass filter. Because of its light sensitivity azobenzene is not as satisfactory a standard as potassium chromate for use in a photoelectric colorimeter.

TABLE 1

Effect of solvent on the light absorption of carotene
(4 mg. per liter, 1 cm. thickness)

Solvent	$\log \frac{I_0}{I}$ Filter 585 + 428	$\log \frac{I_0}{I}$ Filter 556 + 038
Petroleum ether	.531	.735
Ethyl ether	.516	.764
Methyl alcohol	.513	.768
Abs. ethyl alcohol	.499	.792
Cottonseed oil	.470	.756
Melted butter 75°	.47	......
Benzol	.391	.765
Chloroform	.355	.452
Carbon bisulfide	.306	.596

The observed absorption coefficient for our standard carotene in petroleum ether, with filters 556 and 038 was $\log_{10} \frac{I_0}{I} = 0.198$ for solutions of 1 cm. depth and containing 1 mg. per liter. Kuhn (6) gives a value of the molar extinction coefficient of 36×10^4 for beta carotene in hexane and 33×10^4 for alpha. Miller, MacKinney and Zscheile (7) found 32.6×10^4 for both alpha and beta carotene in 20 per cent ether–80 per cent ethanol. These are maximum values for strictly monochromatic light. Judging from the shape of the absorption curve it is probable that where filters are used the observed values should be somewhat lower than 30×10^4. Our value of absorption coefficient can be transformed into molar extinction coefficient by multiplying by $1000 \times 536 \times 2.30$. The result, 24.5×10^4 is close enough to the expected value to serve as an additional check on the method.

PROCEDURE

The actual determination of carotenoid in milk fat is made as follows: The fat is prepared by churning about 100 ml. portions of cream by shaking, then washing with cold water, melting and centrifuging. A 3 to 5 ml. sample is placed in the precision tube and warmed to about 75° C. to produce a clear solution. Another precision tube containing water is inserted in the colorimeter and the diaphragm adjusted until the microammeter reading is 15.0.

The water tube is removed and the tube containing fat is inserted and the reading recorded. The logarithm of the ratio of the reading for water to the reading for melted fat is the absorption coefficient of the melted fat. The carotenoid content can be obtained from the absorption coefficient by reference to a calibration curve as shown in Figure 2. It is not necessary to calculate the absorption coefficient for each determination. A table can be prepared giving carotenoid content in mg. per liter of melted fat from the single readings made on the melted fat if the light is so adjusted that the readings for the water tube are always exactly 15.0.

There are a number of sources of error which limit the accuracy of the method. The most obvious is the purity of the carotene sample used for standardization. Furthermore there is a possibility of oxidation of the carotenoid during the churning and warming of the sample. Also since the color of carotenoids is so sensitive to temperature and change in solvent it is reasonable to expect that the same amount of carotene would produce a slightly different color in different milk fat samples. Actual readings on the instrument deviate from the mean on an average of ± 0.7 per cent. A great deal of further work will be needed to establish the limits of accuracy of the method. The analytical uncertainties are no greater than in the more involved methods of analysis described in the literature. The chief requirement for a method for studying variations in milk fat is rapidity. Since individual samples vary from 1.5 to 21.5 mg. carotenoid per liter of melted fat, an adequate description of the carotenoid content of milk fat samples can best be given in the form of a distribution curve for which a large number of analyses are required.

SUMMARY

Churn 100 ml. samples of cream by shaking; wash the fat with cold water, melt, and centrifuge clear. Transfer 3 ml. to a precision tube absorption cell and warm to about 75° C. to produce a clear solution. Determine the light absorption for blue light in a photoelectric colorimeter and calculate the carotenoid content in mg. per liter of melted fat with the aid of a calibration curve obtained with pure carotene dissolved in cotton seed oil.

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THE CAROTENOID CONTENT OF MILK FAT

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A study of the carotenoid content of milk fat from New York State cows has been made during the year 1936–37 to get additional information on content and the extent of variation with individual cows, seasons, and breeds. Carotenoid content in the fat from 551 milk samples was determined by photoelectric measurement of light absorption in samples of melted fat using Corning glass filters 585 and 428 by the method described in detail in a preceding publication (9). The results of our analyses are shown in Tables 1 and 2, and Figures 1 and 2.

TABLE 1

Carotenoid content of milk fat present in samples of commercial bottled milk collected in various cities in New York State

Date	Number of samples	Carotenoid content mg./liter of fat	
		Range	Average
July 1, 1936	75	4.85–24.6	10.15
Feb. 10, 1937	173	1.54– 7.26	3.77
March 29, 1937	86	1.51– 7.19	3.38
May 29, 1937	79	3.52–21.5	9.38

TABLE 2

Carotenoid content of the milk from individual cows of the Cornell herd

Breed	Barn-silage feed April 7, 1937			June 8, 1937 Summer-pasture feed		
	Number of samples	Carotenoid content mg./liter of fat		Number of samples	Carotenoid content mg./liter of fat	
		Range	Average		Range	Average
Guernsey	13	4.68–12.0	8.50	13	10.4 –20.8	16.4
Jersey	25	4.15–11.5	5.87	21	6.55–14.2	10.5
Brown Swiss	3	5.14– 6.29	5.65	3	3.95–13.6	7.96
Ayrshire	11	1.93– 9.59	4.27	8	5.77– 9.85	6.58
Holstein	20	1.45– 9.42	3.49	17	2.72–10.6	5.78
Herd	72	1.45–12.0	5.42	62	2.72–20.8	9.95

Expressing the carotenoid concentration in mg. per liter of melted fat* at 75° C. we have observed variations from 1.5 mg. per liter to 24.6 mg. For

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* The unit of mg. per liter has been selected because of the colorimetric method of determination which depends directly on concentrations, and eliminates the necessity of measuring or calculating densities. The method yields the value for total carotenoid which in milk fat is about 94 per cent beta carotene and 6 per cent xanthophyll[3].

commercial milks the average seasonal variation was about 300 per cent, *i.e.* from 3.4 mg. per liter on winter barn feed to 10.2 on summer pasture feed.

The seasonal variation in the Cornell herd was not so great, the carotenoid values holding up better in the winter. The breed variation was considerably greater than the seasonal variation in the Cornell herd. For example, Holstein fat in July contained 5.78 mg. carotene per liter as against 16.4 mg. per liter for Guernsey. We have observed other instances of a similar nature where studies on a single herd yielded results different from those with commercial milks. For example, the seasonal variation in riboflavin is less in the Cornell herd than in commercial market milk samples (10).

The curves in Figure 1 are intended to show the uniformity in milk samples. Winter samples were very much more uniform than summer samples. The difference between Guernsey and Holstein was so pronounced that there was practically no overlapping of the curves.

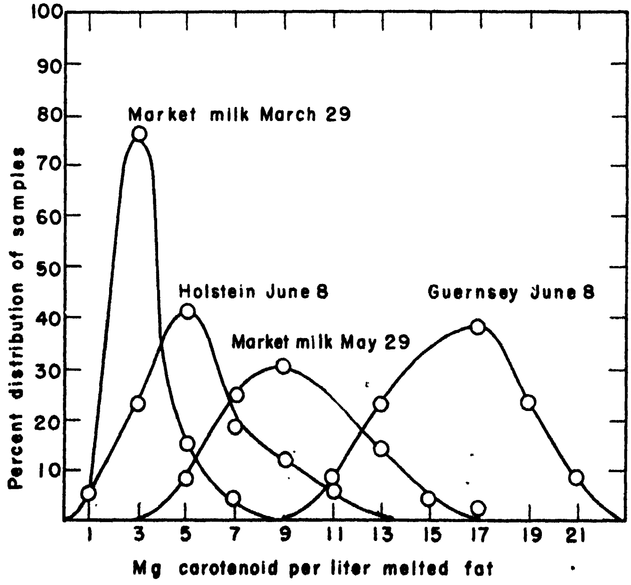

FIG. 1. Distribution of carotenoid in milk fat.

A further study was made on the individual and seasonal variation in carotenoid content of milk fat in a group of 6 cows during the 3 months of May, June and July, 1938. The group contained 3 Guernsey and 3 Holstein cows and were being fed molasses grass silage or, in some cases, phosphoric acid grass silage for the first 5 days. After going on pasture there was a delay of about 1 day before the carotenoid content of the milk fat began to increase. Approximately 2 weeks were required before the maximum value was reached.

During June and July there was no further increase in carotenoid content. The results are shown graphically in Figure 2.

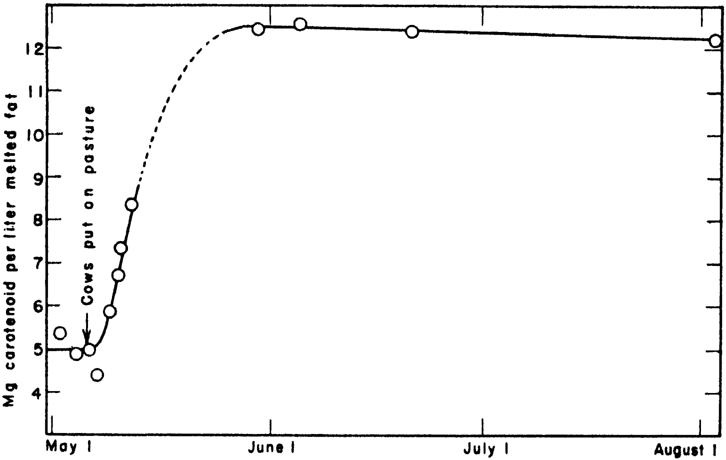

FIG. 2. Seasonal variation in carotenoid content of milk fat.

Our results are consistent with most of the reports in the literature. Gillam (7) studied 70 cows in England and found an average of 0.327 mg. carotene per 100 grams dry butter (2.88 mg. per liter melted butter at 75° C.). His values of 0.15 mg. carotene per 100 grams dry butter for winter feed and 0.6 mg. for moderate grass feed are about one-half as large as the corresponding values for New York State cows. Knudsen and Nielson (11) studied seasonal variations in the color of butter fat, observing the lowest color in April and maximum color in May-June. Baumann and Steenbock (1) report samples of March butter of 2.2 micrograms per gram dry butter and July samples of 8.6 micrograms. These samples are very similar to many analyzed by us. Baumann and Steenbock (2) found among breeds that the Guernsey had the highest carotene content in the fat, that the Holstein and Ayrshire were lowest, and that the Jersey and Brown Swiss were intermediate. We have confirmed this and found, for the cows examined, that Ayrshire milk fat has more carotene than Holstein, and Jersey more than Brown Swiss. The results of Wilbur, Hilton and Hauge (14) are much higher than any other values in the literature, namely, 1.8 mg. per 100 grams fat for Ayrshire and 3.6 to 4.0 for Guernsey. Their determinations were made with a Lovibond tintometer and no actual experimental data were reported.

In going from breed to breed there is no direct relationship between carotenoid content of milk fat and vitamin A potency. A small fraction of the carotenoid pigment is inactive xanthophyll and a large part of the vitamin A potency is due to the colorless vitamin A. Wilbur, Hilton and Hauge (14) found the vitamin A in both Ayrshire and Guernsey milk fat to be practically the same by biological tests. Baumann and Steenbock (2) also reported that breed differences in milk fat with respect to vitamin A activity are small (spectrographic method). Thus on the same feed vitamin A per se is lower in the milk fat from those breeds which are high in carotene. In commercial (mainly Holstein) milk carotene accounts for only 15 per cent of the vitamin A potency (1) but for most of the activity in Guernsey milk. Gillam and others (3, 8) have studied both vitamin A and carotene by the spectrographic method in milk fat from 8 cows of 4 English breeds and they have found large individual variations, but no significant differences between breeds. Davis and Hathaway have observed that whole milk from different breeds is uniform in vitamin A potency (6). Because of the small number of samples investigated in these studies there is some uncertainty in the actual values. Obviously some of the results are contradictory because if there is no difference between breeds with respect to whole milk there must be a difference with respect to milk fat.

As far as individual variations in the same breed are concerned, Treichler, Grimes and Fraps (13) find that highly colored milk fats are apt to be high in vitamin A potency, but not necessarily. Carotene makes up anywhere from 1 to 90 per cent of the potency. Calculations of potency from direct determinations of carotene and vitamin A are somewhat uncertain because 1 mg. of carotene in butter may yield anywhere from 0.16 (3) to 0.6 (5) mg. of vitamin A as measured by activity. It would be valuable to make comparative studies of vitamin A and carotene on a much larger number of cows than any of these workers have studied. Determinations of vitamin A by $SbCl_3$ have been greatly simplified but are still time-consuming. The presence of an inhibitor in summer milk fat prevents development of the color (4) unless the fat is saponified. Methods have been developed for the extraction of carotene and vitamin A from whole milk (12, 15) when the samples are too small for convenient separation of the milk fat.

SUMMARY

The average carotenoid content of the commercial, bottled market milk in New York State was 3.4 mg. per liter of melted fat in winter and 10.1 in summer. The corresponding values for average Holstein milk from the Cornell herd was 3.49 and 5.78; for Jersey milk 5.87 and 10.5 and for Guernsey milk 8.50 and 16.4.

Bottled market milk ranged from 1.51 to 7.26 in winter and from 4.85 to 24.6 in summer. Individual variations within the same breed are much larger than the variations among average values for the different breeds. Individual samples of Holstein milk in June ranged from 2.72 to 10.6, and Guernsey milk from 10.4 to 20.8.

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DIFFERENCES IN ADSORPTION OF SOLID AND LIQUID FAT GLOBULES AS INFLUENCING THE SURFACE TENSION AND CREAMING OF MILK

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This investigation was undertaken to see if there was a reversible difference in adsorption, on the surfaces of solid fat globules, or fat globules which have passed through the solidifying zone of temperature, on the one hand, and liquid fat globules on the other. The factors influencing gravity creaming and cream viscosity, as well as a number of anomolous behaviors of milk and cream may be interpreted as indicating such a difference. Differences in surface tension and gravity creaming were used as indications of the correctness of this hypothesis. It has wide practical applications in the handling and processing of milk and its products, and has been made the basis of many of our investigations during the past several years.

The most definite indication which we found in the literature, of the correctness of this hypothesis, is the report by Hekma (4), who showed that deeper layers of gravity cream were obtained when centrifugally separated skimmilk was used in making recombined milk than when gravity separated skimmilk was used. He believed that the clumping agent was thrown from the surface of the fat globules by the centrifugal force of the separator, and consequently appeared in larger amounts in the centrifugally separated skimmilk. If his experiments were carried out in the usual manner, the gravity skimmilk was removed from fat globules while in the solid state, and the centrifugally separated skimmilk from fat globules while in the liquid state. The difference therefore might be due to the physical state of the fat and not to method of separation.

Burri and Nussbaumer (3) found that previously cooled milk had a lower surface tension at 20° C. than milk which had not been cooled. This observation has been confirmed by Bauer (1), Quagliariello (7), and Mohr and Brockmann (5). Bauer (1) and Mohr and Brockmann (5) showed that the low surface tension produced by cooling could be increased again to approximately normal by warming the milk to 50–60° C.

Mohr and Brockmann (5) conclude that the material adsorbed on the surface of solid fat globules differs from that adsorbed on liquid fat globules.

EXPERIMENTAL

Method of surface tension measurement

All surface tension measurements reported in this paper were made at 25° C. Both room and solution were adjusted to this temperature.

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A wire loop, sealed to one end of a microscope slide, was used to suspend a slide from one arm of a chainomatic balance. The other end of the slide was dipped into the center of the surface of the liquid contained in a beaker 7 cm. in diameter, held on an adjustable support over the pan of the balance.

The surface tension was calculated as being proportional to the weight necessary to pull the edge of a microscope slide from the surface of milk as compared to the weight necessary to pull the same slide from a pure water surface. The surface tension of water was taken as 71.97 dynes at 25° C. The weights required to pull the different slides used from the surface of water ranged from 0.4530 to 0.4662 grams.

The surface tension of each sample of milk was determined with two different slides. Adjustments were made so that the surface broke from each end of the slide at the same time, and when the balance pointer was at zero. The slide was pulled from the surface approximately 30 seconds after stirring the milk. Duplicate determinations with the different slides did not deviate more than 0.10 dynes and most of the deviations were about 0.03 dynes.

Preparation of milk samples

Raw whole milk was divided into three parts and treated as follows:

(1) Original whole milk as a control.

(2) Milk held at 0–5° C. for 2 hours and then separated at 5°–10° C. with the collecting pan warmed slightly to permit the cream to flow. The skimmilk was reseparated.

(3) Milk held at 60° C. for 30 minutes and separated at 60° C.

The effect on surface tension, of warming and cooling of the products obtained above, as well as of whole milk made by recombining the proper amounts of skimmilk and cream, was determined. A list of the products studied is given below:

1. Original whole milk.
2. Milk recombined from 60° C. skimmilk and 60° C. cream.
3. Milk recombined from 5° C. skimmilk and 5° C. cream.
4. Milk recombined from 60° C. skimmilk and 5° C. cream.
5. Milk recombined from 5° C. skimmilk and 60° C. cream.
6. Skimmilk separated at 5° C.
7. Skimmilk separated at 60° C.
8. Cream separated at 60° C.
9. Cream separated at 5° C.

In the recombining, care was taken to prevent churning. The recombined milks, containing the same fat content as the milk used in separation, were prepared as soon as possible after the products were obtained. The cream was always added to the skimmilk. The products were cooled at once in ice water.

Surface tension measurements

After each product had been held in ice water for at least 2 hours, it was warmed to 25° C. and the surface tension was measured. The product was then warmed to 60° C. and cooled to 25° C., and the surface tension was again measured. The entire set of experiments was performed on three different lots of fresh raw milk. The relative effects were almost identical with each lot of milk. The average of the values obtained is presented graphically in Figure 1.

The skimmilk separated at a high temperature had a higher surface tension than the skimmilk separated at a low temperature. The failure of the surface tension of skimmilk to be affected by warming and cooling is in agreement with the investigations of others. However, the cream which was separated at 60° C., and its combination with skimmilk separated at 5° C., showed little alteration in surface tension produced by previous warming or cooling, whereas the cream separated at 5° C., the other recombined milks, and the original milk, showed a difference in surface tension due to previous warming and cooling.

Experiments similar in type to those presented in Figure 1 were carried out, in which the separation was accomplished by centrifuging in glass tubes in a laboratory centrifuge, placed in a cold room in one case and in a warm room in the other. The results were in general agreement with those presented in Figure 1. Therefore, the differences cannot be attributed to the mechanical action or aeration encountered in the ordinary centrifugal cream separator.

The definite differences in surface tension presented in Figure 1 indicate that the adsorbed material on the surface of solid fat globules differs from

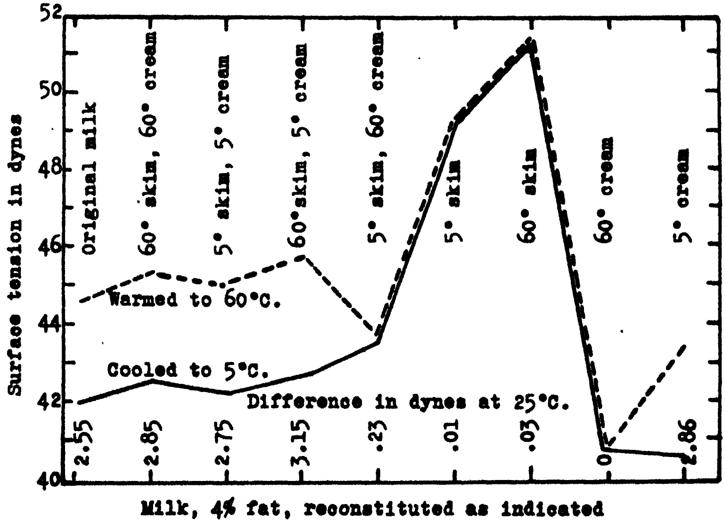

FIG. 1. Surface tension at 25° C. of recombined milk as affected by warming and cooling.

that on liquid fat globules. This is in agreement with our interpretation of the results of Hekma's experiments as being due to a difference in adsorption between the solid and liquid fat globules, and not due to the effect of the separator in freeing the surface, since the cream was separated by the centrifuge in both cases.

Milk which has been heated to temperatures sufficiently high to destroy the clumping property of the fat globules does not show the change in surface tension due to warming and cooling. Heated milk, even after cooling and warming to 25° C., has the surface tension corresponding to warmed milk cooled to 25° C. This renders doubtful Quagliariello's (7) explanation that the lowering of the surface tension produced by cooling is caused by the extrusion of the lower glycerides, due to the solidification of the fat globules.

Gravity creaming as an agglutination process

One of the principal objects of these experiments was to throw light on the process involved in the agglutination of the fat globules, a process which is necessary to obtain gravity creaming of market milk. At the Hoorn Experiment Station in Holland, studies had previously been made of the agglutinating power of various protein fractions obtained from the blood of cattle. Brouwer (2) found that the euglobulin fraction exerted agglutinating power on the fat globules of milk. Sumner, Howell and Zeissig (8) have isolated and crystallized an agglutinin called concanavalin A, from the jack bean, which has power to agglutinate the fat globules of milk if added in fairly large amounts. This material will agglutinate heated milk as well as homogenized milk. Its agglutinating power seems to be more marked when the fat globules of milk are in the liquid state. This temperature effect is apparently the reverse of that of the natural agglutinin of milk, at least in the amounts in which it is present normally.

Our work on surface tension and the investigations cited led us to adopt the following working hypotheses as a partial explanation of the natural clustering which results in the gravity creaming of whole milk:

(1) The clustering of fat globules is due to an agglutinin in the milk.
(2) The agglutinin tends to be more strongly adsorbed, and consequently exerts its greatest action on the solid or solidifying fat globules.
(3) The agglutinin is not adsorbed on liquid fat globules, and consequently does not clump them.
(4) The agglutinin is altered or rendered inactive by heating to high temperatures.

The correctness of these hypotheses has been greatly strengthened by the isolation of the agglutinin which is present in milk in very small amounts, and by the preparation of fractions in which it is almost entirely lacking. The methods of isolation and a study of the properties of this agglutinin will be reported later. The essential correctness of these ideas as applied to natural milk is shown by the following creaming experiments.

In accordance with the hypothesis that the agglutinin is adsorbed on the solid but not on the liquid fat globules, the combination of skimmilk separated at a high temperature (45–60° C.) with cream separated at a low temperature (0–10° C.) shows good creaming properties, while the reverse combination shows poor creaming properties. The recombination of the creams with their own skimmilks shows approximately the natural creaming properties of the original milk. However, a slight decrease in creaming was often found when the products which were separated cold were recombined. After the cream and skimmilk were mixed, the mixture was heated to 45–50° C. and was then cooled in the ice water for creaming. A large number of experiments of this type were carried out. Some typical examples are presented in Table 1.

TABLE 1

Cream volume obtained on milk recombined from cream and skimmilk separated at 45° C. and at 10° C.

Expt. No.	Reconstituted milk made from				Cream volume on milk after holding at 2°–5° C.			
	Cream		Skimmilk					
	Sep. temp. °C.	Heat treat. °C.	Sep. temp. °C.	Heat treat. °C.				
Expt. A—Fat content 6.0%					Hours held			
					3	8	24	48
					%	%	%	%
1	45	Raw	45	Raw	30	28	23	20
2	45	Raw	10	Raw	0	0	15	16
3	10	Raw	10	Raw	26	23	20	18
4	10	Raw	45	Raw	28	25	22	20
Expt. B—Fat content 5.8%					Hours held			
					2	4	24	
					%	%	%	
5	45	Raw	45	Raw	32	28	22	
6	45	Raw	10	Raw	0	0	18	
7	10	Raw	10	Raw	26	24	18	
8	10	Raw	45	Raw	30	27	21	
9		Original	milk		24	22	18	
Expt. C—Fat content 4.0%					Hours held			
					4	12	24	36
					%	%	%	%
10	45	Raw	45	Raw	17	13	13	11
11	45	70	45	Raw	17	13	13	11
12	45	Raw	10	Raw	0	8	8	9
13	45	70	10	Raw	0	7	7	8
14	10	Raw	45	Raw	20	15	14	12
15	10	70	45	Raw	18	14	13	12
16	10	Raw	10	Raw	16	14	13	12
17	10	70	10	Raw	8	10	10	10
18	45	Raw	45	70	0	6	6	7
19	45	Raw	10	70	0	2	3	5
20	10	Raw	45	70	10	11	10	10
21	10	Raw	10	70	10	11	10	10

Evidence was presented by Van Dam and Sirks (9), Hekma (4), and Palmer, Hening and Anderson, (6), that the agglutinating agent resides in the plasma. The theory presented here indicates that plasma is largely responsible for the clumping only when the milk is separated while the fat is in the liquid state. This is a confirmation of previous work, but indicates that the plasma investigated by previous workers was obtained while the fat was in the liquid state. If the plasma is separated from the fat while the fat globules are in the solid state, then the fat globules may actually exert a more important influence on creaming than the plasma. This is indicated in Table 1 by the effect of heating some of the samples of cream and skimmilk before recombining them. Paired experiments 10–11 and 12–13 indicate that heating the cream did not appreciably reduce the cream layer formed on the recombined milk. Reductions due to heating the cream were found, however, if the cream was separated while the fat globules were solid, as indicated by pairs 14–15 and 16–17. The most definite effect, however, is shown in experiments 18–21, in which heated skimmilk when combined with

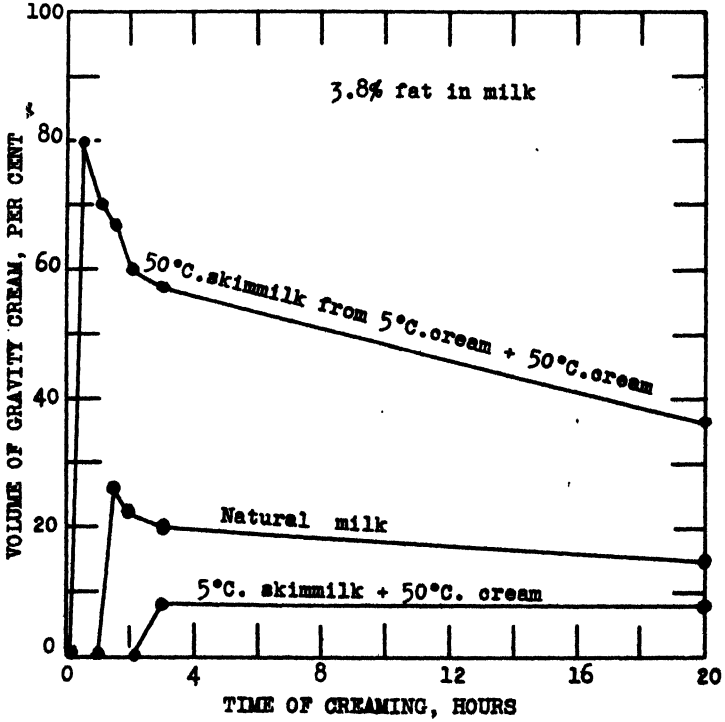

FIG. 2. Gravity creaming of whole milk made by recombining cream with skimmilk which had been depleted and with skimmilk which had been enhanced in agglutinin content.

cream separated at 45° C. formed little cream, while heated skimmilks, recombined with cream separated with the fat globules in the solid state, showed appreciable creaming.

These experiments indicate obvious methods of concentrating the agglutinating material. One procedure used, which tends to bear out the correctness of our hypothesis, is the following: Cream was allowed to rise on cold, raw milk. The skimmilk was withdrawn from beneath the cream layer and the cream was then concentrated by separation at 5–10° C. The cream was then warmed to 50° C. and separated again, so that what might be called 50° C. cream plasma was obtained. The separation of the cream while the fat was in the solid state concentrated probably three-fourths of the agglutinin of the milk in the cream. When the cream was heated to 50° C. the fat surface was freed of the agglutinin which then appeared in the plasma phase. The same high-temperature-separated cream was used to produce recombined 3.8 per cent fat milk from 50° C. cream plasma and from the skimmilk obtained originally in the first cold separation. The results of a typical experiment are presented in Figure 2. The cream layers obtained from milk recombined from 50° C. cream plasma were truly remarkable. They formed 10 minutes after the milk was cooled. The layer when first formed occupied from 80 to 90 per cent of the volume, and after standing cold for 20–24 hours the cream volume was usually 35–45 per cent. The usual reduced values were shown by the cream volumes obtained when the skimmilk, separated while the fat globules were solid, was used in recombining.

DISCUSSION

The effect of the physical state of the fat globules at the time of separation, on the properties of the skimmilk, whey and cream; have been studied in connection with foaming, churning, cream whipping, lipase activity, creaming and surface tension. This article is limited to some of the effects on creaming and surface tension.

In natural milk, presence of the agglutinin in the plasma phase is apparently associated with an increase in surface tension and liquefaction of the fat; its absence from the plasma phase or presence on the surface of the fat globules is associated with a lower surface tension and the solidification of the fat. Whole milk containing the active agglutinin shows the effect of previous warming and cooling, on the surface tension. The high-temperature-separated skimmilk containing the agglutinin has a higher surface tension than does the low-temperature-separated skimmilk in which the agglutinin is largely absent. Cream separated while the fat is in the solid state contains the agglutinin and consequently shows the effect of previous warming and cooling on surface tension. Cream separated while the fat is in the liquid state contains little agglutinin, and does not show the effect of previous warming and cooling on the surface tension. The combination of 5° C. skimmilk and 60° C. cream has a relatively low surface tension, contains little agglutinin, and shows little effect of previous warming and cooling, on the surface tension.

Whether the agglutinin is actually the material which influences the surface tension is not established. One would expect that, if the agglutinin were liberated to the plasma phase and the surface tension were affected at all, it would be lowered. Protein systems usually do not meet all of the requirements demanded in the strict application of the adsorption theory as advanced by Gibbs. Such an effect is therefore not impossible. There are, however, other possibilities.

A displacment of substances on the surface of the fat globule may occur. When the agglutinin is adsorbed on the surface of the fat globules a surface-tension-reducing substance may be displaced, which then appears in the plasma phase and lowers the surface tension. When the agglutinating agent is liberated from the fat surface the surface-tension-reducing substance may be again adsorbed on the fat. This last hypothesis is a little like that proposed by Quagliariello (7), but involves a heat labile protein as one component and thus the exchange would be stopped by heating. It gains support from the fact that the skimmilk separated at 5° C. has poor foaming properties, while skimmilk separated at 50° C. has marked foaming properties; and from the fact that when low-temperature-separated skimmilk is extracted with petroleum ether its foaming properties then approach those of the high-temperature-separated skimmilk. The difference between the two types of skimmilk can hardly be due to less efficient separation at the lower temperature, because repeated separations of the skimmilk at higher temperatures do not alter the relationships.

There are several other possible explanations.

CONCLUSIONS

1. Adsorption on the surfaces of liquid and of solid fat globules in normal unheated milk is different.

2. The difference in adsorption due to the difference in the physical state of the fat markedly affects surface tension, foaming, and gravity creaming.

3. The clumping of the fat globules which results in gravity creaming is produced by an agglutinating substance.

4. The agglutinating substance is adsorbed on the surface of the solid or solidifying fat globules and not on the surface of the liquid fat globules.

5. The agglutinating substance is concentrated in cream separated at low temperatures and is relatively absent in the corresponding skimmilk, and it is relatively low in cream separated at high temperatures but is present in the corresponding skimmilk.

6. The concentration of the agglutinating substance can be greatly increased by first separating cream at a low temperature and then separating it

to a higher fat content at a higher temperature at which the fat is liquid. The cream plasma obtained at this higher temperature is very active in agglutinating power.

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THE RIBOFLAVIN VALUE OF MILK[1]

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INTRODUCTION

Milk is recognized today as a foremost source of riboflavin (vitamin G) in human dietaries. It is desirable, therefore, that our knowledge of the variations of this important constituent of milk be as extensive as possible. Hunt and associates (10, 11, 12) studying the influence of the ration on the vitamin G content of milk reported better rat growth on milk from cows on pasture than on milk from cows on dry feed. Whitnah and associates (18), using the rapid fluorimetric method, have reported on the riboflvain content of milk samples collected in different months and correlated with other constituents of the milk.

A recent study, reported from this laboratory by Kramer and coworkers (13), showed significant variation, as determined biologically, in the riboflavin value of milk produced during the first month of lactation. The present investigation is a continuation of this study and its purpose was to secure information concerning the riboflavin value of milk produced under different well defined herd feeding conditions.

PROCEDURE

Samples of milk (Table 1) for these experiments were secured from the Kansas Agricultural Experiment Station herd. Twenty-four hour collections of milk were made and composite samples retained for riboflavin determinations. All samples of milk were held below 0° C. until used. A description of the samples used and types of rations fed appears in Table 1.

For the biological assays, the Bourquin-Sherman (6) method was used. This method has been shown by Bisbey and Sherman (5) and Bessey (4) as reliable for quantitative estimation of the flavin content of foods. The samples of milk were fed as supplements to the basal diet at the rate of 3 grams per day for 6 days a week. The standard, fed for reference to the positive controls, was riboflavin, PX grade, secured from the Biological and Chemical Laboratories of the Borden Company, Research Division. It was

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TABLE 1

Riboflavin determinations of milk

Milk samples						Biological assay							Fluorimetric determinations		
						Rats used					Estimated riboflavin				
Sample		Date of collection	Sample number	Fat	Total solids	Daily portion	Number	Average time depleted	Average initial weight	Average gain	Per portion	Per gram milk	Riboflavin per gram milk	Variation from biological value per gram	
				%	%	gm.		Days	gm.	gm.	Micro-grams	Micro-grams	Micro-grams	Micro-grams	%
	7 Holstein cows														
1	Herd ration, no pasture	9– 3–36	1	3.5	12.07	3	10	13	49	40	6.0	2.0	1.76	− 0.24	− 12
2	Herd ration, fall pasture	12– 3–36	2	3.1	11.72	3	10	12	47	50	7.5	2.5	1.88	− 0.62	− 25
	5 Jersey cows														
3	Herd ration, no pasture	9– 3–36	3	4.5	13.89	3	10	13	48	43	6.5	2.2	2.3	+ 0.10	+ 4
4	Herd ration, fall pasture	12– 3–36	4	5.6	15.30	3	10	13	47	56	8.5	2.8	2.55	− 0.25	− 9
	4 Holstein cows														
5	Herd ration, no pasture	4–14–37	5	3.2	11.5	3	10	16	49	31	4.8	1.6			
6	Herd ration, spring pasture	5–12–37	6	3.3	11.8	3	11	15	49	31	4.8	1.6			
	4 Jersey cows														
7	Herd ration, no pasture	4–14–37	7	5.2	14.67	3	10	15	48	40	6.0	2.0			
8	Herd ration, spring pasture	5–12–37	8	5.1	14.53	3	10	15	50	45	6.8	2.3			
	4 Guernsey cows														
9	Herd ration, no pasture	4–14–37	9	5.9	14.21	3	10	15	49	44	6.6	2.2			
10	Herd ration, spring pasture	5–12–37	10	5.2	14.50	3	10	15	50	40	6.0	2.0			
	7 Holstein cows														
11	Experimental ration	9– 9–36	11	4.0	11.21	3	10	13	46	46	7.0	2.3	2.20	− 0.10	− 5
	Station herd														
12	Herd ration	1–15–37	12	4.0	12.75	3	13	16	45	40	6.0	2.0			
						micro-grams									
	Negative controls					0	19	14	46	0					
	Controls for reference curve														
	Riboflavin					2.5	10	13	46	17					
	Riboflavin					5.0	9	14	49	33					

Description of samples used:

Nos. 1, 3, 5, 7, 9 herd ration of alfalfa hay, silage and grain.
Nos. 2, 4 rye pasture—Holsteins, 2 weeks—Jerseys, 3 weeks.
Nos. 6, 8, 10 spring pasture for 3 weeks.
No. 11 experimental ration, No. 2 upland prairie hay and grain without green feed or pasture for over two years.
No. 12 winter milk from college herd representing four breeds on herd ration of alfalfa hay, silage and grain.

fed at two different levels; 2.5 micrograms and 5 micrograms, 6 days per week. For the biological assays, all groups but one contained at least 10 rats. The animals (Wistar Institute strain) were confined in cages having raised wire screen floors and checked daily for evidence of coprography.

A number of the milk samples were also assayed fluorimetrically using the method developed by Whitnah and associates (17).

DISCUSSIONS

The results are presented in Table 1. Although the feeding tests extended over a period of ten months, the groups of rats showed satisfactory uniformity as indicated by the average lengths of depletion periods and average initial weights. The composite gain curves (Figure 1) show that sup-

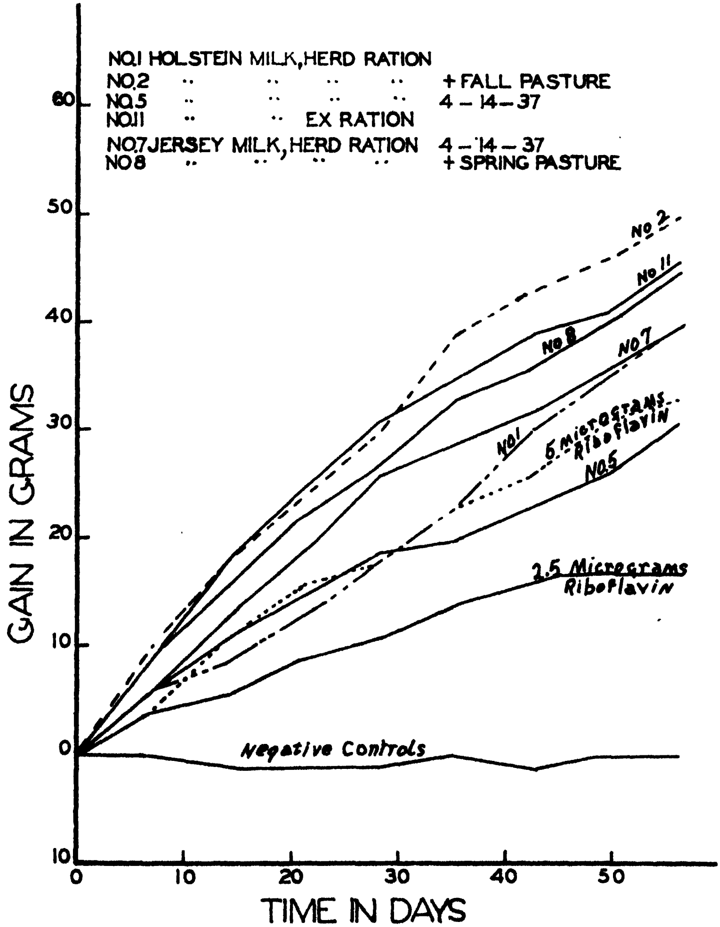

FIG. 1. Average gains of rats fed milk and riboflavin supplements as indicated.

plements produced regular growth response throughout the 8-weeks experimental period. A flattening of the curve during the last two weeks of feeding is observed for the smallest supplement, namely, 2.5 micrograms riboflavin. However, this curve compares well with that of the rats receiving twice the amount of this supplement. The total gains for the 8-weeks period on these two levels were 17 and 33 grams respectively, which is almost in the ratio of 1 to 2. These riboflavin curves, therefore, appear reliable for reference, since the growth response was almost in direct proportion to the amount fed. The average gain per week in each case checked with the curve presented by Ansbacher and associates (1).

The riboflavin content of a daily portion of each milk sample was estimated both by comparison of the composite gain curves with the reference curves and also by direct proportion between total gains for eight weeks as compared with total gains shown on the reference curves. These estimates were made on the assumption that growth responses from larger supplements were in direct proportion to the riboflavin content. Ansbacher, Supplee, and Bender (1) have suggested that the response diminishes as the supplements become large. However, none of the portions here used produced gains sufficient to reach this upper level of diminishing returns. Only two 8-weeks gains, those of 50 and 56 grams (Table 1) seemed large enough to be questioned. Values estimated from these gains were checked fluorimetrically, so the biological values may be considered reliable. The estimated riboflavin values are expressed as micrograms per gram of milk.

Some of the estimates of riboflavin content based on biological data were compared with values obtained from readings with the Eveready Fluoray lamp and are in reasonable accord with these values. In no case were the fluorimetric readings excessively high; rather, they tended to be lower than the estimates derived from biological data. The maximum difference between values determined by the two methods was 25 per cent with an average difference of 10 per cent.

Biological assay of samples No. 1 and No. 3, collected in September at the end of an abnormally dry summer, indicated the presence of at least 2 micrograms of riboflavin per gram of milk. Later samples (No. 2 and No. 4) collected in December after the cows had access to good pasture for periods up to one month, gave values approximately one-fourth higher. On the other hand, samples No. 6, 8, and 10, collected from other groups of cows on pasture the following spring did not show the expected increase when compared with milk samples collected from the same cows near the end of the winter feeding period (No. 5, 7, and 9). Only in the case of the Jersey composite (No. 8) was a small increase observed in the riboflavin content of the milk which might be attributed to the influence of the supplemental pasture. The Holstein sample (No. 6) showed no increase in riboflavin as a result of pasture feeding, while the biological assay of the milk from the group of Guernseys (No. 10) actually gave a small decrease.

It should be emphasized that throughout the Central Plains area the summer of 1936 was one of the driest on record with abnormally high summer temperatures prevailing. The station herd was without green feed from June until October, the normal pasture season, and barn feeding of the winter herd ration was followed. The lack of pasture combined with the excessively hot weather (60 days with temperature readings 100° F. or over) represented extreme conditions which undoubtedly had its effect on the physiological behavior of the cows involved. Regan and Richardson (14) have studied the effect of environmental temperature on high producing cows in a large psychrometric room with air movement and relative humidity closely controlled. These workers observed that as the temperature was elevated above the pyrexial point (80 to 85° F.) an alteration occurred in the characteristics of the milk produced which included a lowering of the casein and solids-not-fat content. In the present experiment the milk production of the cows was lowered as a result of the extreme heat and it is possible that the secretion of riboflavin was also affected, to return to more normal levels, possibly even with some increase, after a period of cooler weather and access to pasture. This is somewhat conjectural and offers little aid in explaining the inconsistent results obtained the following spring. Similar behavior has been observed with respect to the vitamin C in milk which is apparently more responsive to seasonal and climatic changes than to the feed which the cow receives. Bechdel and associates (2, 3) have found that the vitamin B complex may be synthesized by microorganisms in the rumen of the cow and that the amount in the milk is not dependent on the amount in the ration. Gunderson and Steenbock (9) showed that milk produced on pasture and fresh green alfalfa did not contain any more vitamin B (complex) than milk produced on silage and dry feed. These workers observed that increasing the vitamin B intake by feeding wheat germ had no discernible effect both absolutely and in relation to the amount of milk secreted on the vitamin B content of cow's milk. While it is not possible to interpret the foregoing work in terms of riboflavin, which is one of the ensemble of known B factors, it does appear to support the conclusion that the ration is not a major factor influencing the riboflavin content of milk.

A statistical analysis of all results in the present study grouped with respect to before and after pasture feeding and involving growth records of some 50 rats in each instance showed no significant increase which might be attributed to the addition of pasture to the ration. A difference of 0.2 ± .18 was obtained which is less than would ordinarily be considered as significant.

In these experiments Jersey and Guernsey samples gave slightly higher estimated riboflavin values than comparable samples of Holstein milk. Attention is called to the fact that the milk produced by the Holsteins on the restricted ration (dry lot for over two years) was at least as rich in riboflavin as samples No. 1 and No. 3 and as the sample of winter milk (No. 12) from the station herd. In terms of total milk production, the Holstein cows on the restricted ration, because of their average milk yield, produced only approximately half as much riboflavin per cow per day as did the Holsteins in the station herd. Also, because of higher daily milk yield these Holsteins produced greater average daily yields of riboflavin than the Jerseys or Guernseys.

The growth records of the rats receiving 2.5 and 5.0 micrograms riboflavin in the present investigation would indicate that a Bourquin-Sherman unit is equivalent to about 3.6 micrograms riboflvain. Estimates ranging from 2.0 to 4.0 micrograms of riboflavin equivalent to one Bourquin-Sherman unit have been reported in the literature (4, 7).

Euler and Adler (8) have estimated that milk contains 2 to 3 micrograms of riboflavin per ml. In the present study an average riboflavin value for all milks tested indicated 2.1 micrograms per gram with a range of 1.6 to 2.8 micrograms. On this basis a quart of this milk would furnish approximately 542 Bourquin-Sherman units, which is only slightly less than the tentative figure of 600 units used at present in determining the adequacy of human dietaries.

SUMMARY AND CONCLUSIONS

Biological assays of samples of milk secured in September, 1936, following an abnormally dry summer with no green pasture available, showed riboflavin values of about 2 micrograms per gram of milk. Later, after the cows had been on pasture, values approximately 25 per cent higher were secured. However, data obtained the following spring did not show the expected increase as a result of pasture feeding when compared with values on the winter ration. When all values representing before and after pasture feeding were submitted to statistical analysis no significant difference was found. It is concluded, therefore, that under the conditions of this study the rations did not have any very significant effect on the riboflavin content of milk. It is suggested that climatic and seasonal factors may be of more importance. In further support of this is the fact that the milk from the experimental herd maintained without green feed for over two years yielded milk at least as rich as the milk from the station herd, presumably fed a more desirable ration.

The average riboflavin value for all samples of milk tested was 2.1 micrograms per gram of milk. On this basis a quart of milk would furnish approximately 542 Bourquin-Sherman units.

The riboflavin determinations of the milks by a fluorimetric method gave results valuable as checks and comparable to biological assay findings. The maximum difference between the biological findings and the Fluoray lamp readings was 25 per cent in a limited number of determinations with an average difference of ± 10 per cent. While consistently lower the fluorimetric results showed the same general trend.

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WATER SORPTION BY DRY MILK SOLIDS. II. THE RELATION BETWEEN VOLUME CONTRACTION AND THE DEGREE OF SORPTION

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The first paper of this series (1) has shown the extent of volume contraction of dry milk solids, prepared by different processing treatments, when exposed to a large amount of water. The present paper deals with the volume contraction exhibited by the dry milk solids at different degrees of sorption. The maximum volume contractions as reported represent values characteristic of the different samples, and it is important to know the water content to which these are equivalent. According to certain workers (2, 3), volume contraction accompanying water sorption represents a decrease in the specific volume of water of 30–40 per cent. This means a volume contraction of 3–4 cu. mm. per gram of sorbed water. Whether these values are applicable to dry milk solids is not known. The data to be presented here will establish this relationship.

EXPERIMENTAL

The changes occurring when small quantities of water are added to a large amount of solids are difficult to measure accurately. It was decided, consequently, to measure the volume contraction of the solids after they had been allowed to sorb predetermined quantities of water. From values thus obtained the volume contraction occurring in dry milk solids of definite water content can be determined; and the extrapolation of these data will give the maximum volume contraction occurring at zero water content of the sample, together with the water content above which no volume contraction will occur.

The samples used were from the same source as those reported previously (1). They consist of eight samples of dry milk solids, identified in Table 1.

TABLE 1

Identification of samples

Sample no.	Preheat temperature 30 minutes		Manufacturing process
1	71.1° C.	160° F.	spray
2	76.6	170	
3	82.2	180	
4	93.3	200	
5	71.1	160	atm. roll
6	76.6	170	
7	82.2	180	
8	93.3	200	

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Volume contraction was measured as previously described (1, 4). To determine how the water content affects the volume contraction, each sample was made up to a definite water content by adding a known quantity of water to a weighed amount of dry milk solids; by allowing sorption to take place for 24 hours, since the previous results had indicated that sorption was usually complete within that time, and then by measuring the residual volume contractability of the system.

RESULTS

The results are expressed as volume contraction (C), representing cubic millimeters per gram of solids; and as degree of sorption (i), representing $\frac{\text{weight of water sorbed}}{\text{weight of solids}} \times 100$—in other words, the sorbed water expressed as percentage of the dry material.

Table 2 shows the volume contraction (C), of each sample after being made up to different water contents:

According to these data, after a sample has been allowed to sorb a quantity of water, the extent of volume contraction is less than before and is further

TABLE 2

The volume contraction of dry milk solids of different water contents

Water content per cent of dry material	Sample No.							
	1	2	3	4	5	6	7	8
2.0	....	....	63.4	64.2	....	....	38.0	42.5
2.1	....	98.0	....	....	....	....	....	....
2.4	75.8	....	....	....	....	....	....	....
2.5	....	....	....	51.0	....	50.0	....	....
2.9	....	....	53.0	....	30.2	....	....	35.2
3.8	....	....	....	....	....	....	30.2	....
4.4	58.0	....	....	....	....	....	....	....
5.0	....	....	....	....	21.8	36.0	....	27.5
6.4	53.0	....	....	....	....	....	....	....
7.1	....	59.0	....	....	....	....	....	....
7.5	....	....	....	....	....	11.2	17.4	20.0
8.4	47.0	....	....	....	16.0	....	....	....
10.0	37.2	....	31.0	31.0	....	....	....	....
11.0	....	....	....	....	8.3	....	....	....
12.0	....	....	....	....	....	....	7.1	....
12.1	....	38.0	....	....	....	....	....	....
12.4	31.0	....	....	....	....	....	....	....
12.5	....	....	....	....	....	4.3	....	10.6
13.0	....	....	....	....	4.3	....	....	....
15.3	....	....	....	....	....	....	2.1	....
17.1	....	26.1	....	....	....	....	....	....
17.3	....	....	....	8.5	....	2.0	....	....
18.0	....	....	7.5	....	....	....	....	....
19.7	11.0	....	....	....	....	....	....	....
20.0	....	....	5.0	....	....	....	....	....
22.9	....	....	....	2.0	....	....	....	....
27.1	....	10.9	....	....	....	....	....	....
27.5	2.0	....	....	....	....	....	....	....
32.1	....	6.7	....	....	....	....	....	....
33.9	....	2.8	....	....	....	....	....	....

reduced as the quantity of water pre-sorbed is increased. From these data one should be able to construct curves and, by extrapolating to the axes, to obtain reasonably accurate values for the volume contraction (C) at 0 per cent water and for the degree of sorption (i) above which no further contraction will occur. Since the curves are nearly tangent to the ordinate and abscissa at these values, the exact point of intersection is difficult to determine; but if the logarithms of these values are plotted, curves result which, when extrapolated, intersect the axes nearly at right angles, thus permitting reasonably accurate location of the zero points. Curves thus obtained appear in Figure 1.

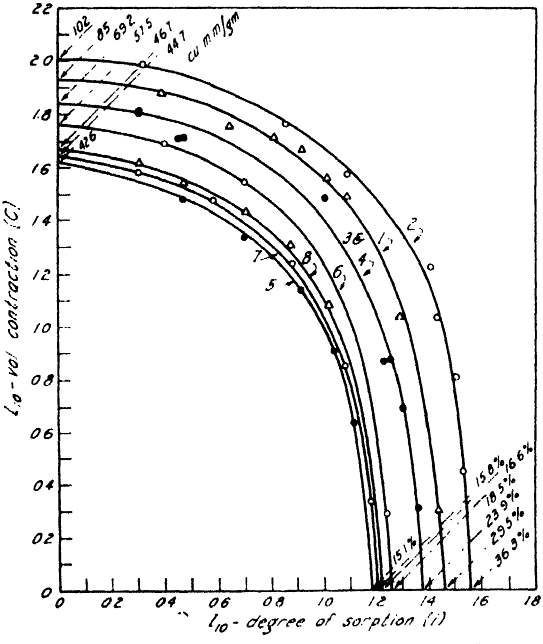

FIG. 1. Logarithmic values of volume contraction at different degrees of sorption.

As will be observed, the maximum volume contraction ranges from approximately 42 cubic mm. per gram of solids to 102 cubic mm.; and these values correspond to approximately 15 per cent sorption up to 36 per cent sorption if the extrapolations are assumed to be valid. Pyenson and Dahle (5), using the cryoscopic method, have found that dry milk solids prepared by the vacuum drum process "bind" 61.1 per cent of water when fresh, 40.3 per cent when four weeks old, and 40.1 per cent when eight weeks old if stored in a closed container.

Table 3 shows the degree of sorption (i) corresponding to the maximum volume contraction (C) as obtained by extrapolation, together with the ratio between them in terms of cubic millimeters per 1 per cent water for the different samples.

TABLE 3

The maximum volume contraction and maximum degree of sorption of different samples of dry milk solids

Sample No.	C	i	C/i
1	85.0	29.5	2.87
2	102.0	36.3	2.81
3	69.2	23.9	2.89
4	69.2	23.9	2.89
5	42.6	15.1	2.83
6	57.5	18.5	3.10
7	44.7	15.8	2.83
8	46.7	16.6	2.82
Average	....	....	2.88 ± .06

As this table reveals, the degree of sorption for the differently processed samples is greatest for those preheated at 76.6° C. (170° F.). The effects of excess heat are shown in the lower values for the samples prepared by the roller process and by preheating above 76.6° C. (170° F.) for the spray process. These data express quantitatively the effects of the processing treatments shown previously (1).

DISCUSSION

The experimental value of the constant found for the volume contraction per unit of water sorbed by dry milk solids (namely, 2.88 ± .06 cubic mm. per one per cent water) falls somewhat below the expected value of 3–4 cubic mm. per 1 per cent water. Several reasons may be suggested. Syneresis resulting from the aging for 24 hours may be ruled out because the maximum volume contraction (C) is determined from dry or nearly dry material; if syneresis should occur at higher water contents, then the values for degree of sorption would be too low. Larger values for degree of sorption with the same values for maximum contraction would result in a constant even farther away from the expected value.

A more logical explanation lies in the possibility of lactose hydration. Sucrose, as is well known, forms a hexahydrate in solution (6). Leighton (7) suggests from his studies that lactose acts like sucrose in solution and forms a hexahydrate. Although data on the point are fragmentary, those given in the International Critical Tables for freezing-point depressions (8) indicate some degree of hydration of lactose in solution. Hudson (9) believes that only the alpha equilibrium form is hydrated in solution. It seems reasonably conclusive that lactose molecules in solution are hydrated to a greater or less degree.

Evidence regarding the volume changes resulting from solution of lactose is more positive. The Associates of Rogers (10) cite evidence to show that lactose in 54 per cent solution exhibits a volume contraction of 11 cubic millimeters per gram of lactose. Schmoeger's data (11) on densities of different concentrations of lactose also indicate a volume contraction at the relatively higher concentrations, as is evidenced by the fact that the determined densities exceed the theoretical at concentrations of 10 per cent lactose and above. Below this in the range of 2–5 per cent lactose, however, the measured density of the solutions corresponds almost exactly to the theoretical. Also, experiments with the apparatus used in these studies with the same concentrations of lactose as in the dry-milk studies—namely, 2–3 per cent—have failed to reveal any significant volume change. Possibly—to judge from this evidence—some of the sorbed water is used in hydrating the lactose in solution but does not manifest itself in volume contraction, thus resulting in a volume contraction/degree of sorption ratio smaller than the expected value.

SUMMARY

1. The volume contraction of dry milk solids prepared by different processing treatments was determined after different amounts of water had been added to the dry material.

2. From these data, curves were constructed which, when extrapolated to zero water concentration and zero contraction, gave values for maximum volume contraction and for maximum degree of sorption for each sample.

3. The maximum volume contractions at zero water concentration ranged from 42.6 cubic mm. per gram of dry material to 57.5 cubic mm. for the atmospheric roll-process samples, and from 69.2 cubic mm. per gram to 102.0 cubic mm. for the spray-process samples. These values correspond to degrees of sorption, expressed as percentage of the weight of the solids, respectively, as follows: 15.1 per cent, 18.5 per cent, 23.9 per cent and 36.3 per cent.

4. The volume contraction/degree of sorption ratio was found to be 2.88 ± .06 cubic mm./1 per cent sorption, which compares with the expected value of 3–4 cubic mm./1 per cent sorption. It is suggested that the reason for the low value for this ratio lies in the hydration of lactose in solution, with no accompanying volume contraction within the limits of concentration used here.

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THE DAIRY INDUSTRIAL FELLOWSHIP RESEARCH PROGRAM

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This article is a report of the Dairy Industrial Fellowship Research Program, sponsored jointly by the Dairy Industries Supply Association and the American Dairy Science Association. This report 1, offers a permanent record of the program and 2, gives to the dairy industry an account of the research undertaken and completed under the scholarship grants.

The program was formulated in 1929. At that time Professor R. B. Stoltz and other members of the Dairy Products Judging Committee of the American Dairy Science Association met with Messrs. Schantz, Everett, Breece and other representatives of the Dairy Industries Supply Association, Inc. to discuss the possibility of a joint program, sponsored by the two organizations, for furthering the student dairy products judging program. From this and subsequent meetings there evolved the plan of holding the National Dairy Products Judging Contest as a part of the Dairy Industries Exposition, under the joint sponsorship of the American Dairy Science Association and the Dairy Industries Supply Association, Inc.

The Dairy Industries Supply Association has furnished the financial aid and material assistance necessary for conducting the judging contest itself, and, in addition, has awarded annually, from 1930 to 1937 inclusive, six fellowship grants to six team members or alternates. For 1938 the number of grants was reduced from six to three. The fellowships originally carried a stipend of $750.00, which in 1934 was reduced to $600.00. The number and value of the fellowship grants are given in Table 1.

TABLE 1

Fellowship grants of the Dairy Industries Supply Association, Inc., 1930–1938

Year	Number of grants	Value of each	Total
1930	6	$750.00	$4,500.00
1931	6	750.00	4.500.00
1932	6	750.00	4,500.00
1933	6	750.00	4,500.00
1934	6	600.00	3,600.00
1935	6	600.00	3,600.00
1936	6	600.00	3,600.00
1937	6	600.00	3,600.00
1938	3	600.00	1,800.00
Total	51	....	$34,200.00

These fellowships have made it possible each year for six students to continue their studies in dairying in the graduate school of another college

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for a 12-month period. The Industrial Fellow was thus required to matriculate in the graduate school as a candidate for the degree of Master of Science which necessitated the pursuit of subjects of a major and of a minor nature to dairying and the completion of a research problem closely allied to the dairy industry.

Up to 1934 the Industrial Fellowships were awarded to qualified students who had the highest standing in the judging of all dairy products. Qualification consisted in scholastic standing and in senior ranking at the college, thus enabling the Fellow to begin graduate work the following academic year. Since 1934, the awards have been made to the six leading colleges in the judging of all products. The Fellows were then selected from the members of the winning teams and alternates by the heads of the departments with the counsel of various staff members. The Fellows were permitted to choose the school in which to undertake graduate work from a list of schools represented in the current dairy products judging contest; the ranking individual having first choice and so on.

The problem subject, drawn up by the department of dairying in which the graduate study was undertaken, was submitted to committees representing the American Dairy Science Association and the Dairy Industries Supply Association, Tables 2 and 3, for comment and approval. At the termination of the graduate period a full report of the research undertaken, suitable for publication in the various trade or scientific journals, was required. The acceptance of this report terminated the agreement between the college admitting the Fellow and the donors of the fellowship grant.

TABLE 2

Dairy products judging contest committees, 1926–1939, American Dairy Science Association

1926–27	1927–32	1932–34
R. B. Stoltz, Ch.	R. B. Stoltz, Ch.	R. B. Stoltz, Ch.
William White	William White	William White
M. Mortensen	H. W. Gregory	H. W. Gregory
G. D. Turnbow	G. D. Turnbow	P. A. Downs
H. W. Gregory	P. A. Downs	H. A. Bendixen
1934–36	1936–37	1937–38
H. W. Gregory, Ch.	H. W. Gregory, Ch.	H. W. Gregory, Ch.
William White	William White	William White
P. A. Downs	R. B. Stoltz	P. A. Downs
P. S. Lucas	P. A. Downs	M. J. Mack
R. B. Stoltz	R. W. Smith, Jr.	G. M. Trout
1938–39		
G. M. Trout, Ch.		
William White		
P. A. Downs		
M. J. Mack		
E. L. Fouts		

TABLE 3

Contest and fellowship committees, 1930–1939. Dairy Industries Supply Association, Inc.

1930

K. W. Schantz, Ch., K. W. Schantz Co.
Loomis Burrell, Cherry-Burrell Corp.
E. W. Chandler, The Creamery Package Mfg. Co.
Prof. H. F. Judkins, Representing International Assn. of Ice Cream Manufacturers
Dr. C. I. Corbin, Representing International Association of Milk Dealers
Dr. N. W. Hepburn, Representing American Butter Institute
Roberts Everett, Executive Vice President, Dairy Industries Supply Association, Inc.

1931

K. W. Schantz, Ch.
Loomis Burrell
G. E. Wallis, The Creamery Package Mfg. Co.
Prof. H. F. Judkins
Dr. C. I. Corbin
Dr. N. W. Hepburn
Roberts Everett

1932

K. W. Schantz, Ch.
Loomis Burrell
G. E. Wallis
Prof. H. F. Judkins
Dr. C. I. Corbin
Dr. N. W. Hepburn
Roberts Everett

1933

K. W. Schantz, Ch.
Loomis Burrell
G. E. Wallis
Prof. H. F. Judkins
Dr. C. I. Corbin
Dr. N. W. Hepburn
Roberts Everett

1934

Loomis Burrell, Ch.
G. E. Wallis
W. H. Breen, York Ice Machinery Corp.
Prof. H. F. Judkins
Dr. C. I. Corbin
Dr. N. W. Hepburn
Roberts Everett

1935

G. W. Putnam, Ch. The Creamery Package Mfg. Co.
Loomis Burrell
W. H. Breen
Dr. C. I. Corbin
Prof H. F. Judkins
Dr. C. W. Larson, Representing American Butter Institute
Roberts Everett

1936

G. W. Putnam, Ch.
Loomis Burrell
W. H. Breen
Dr. C. I. Corbin
Prof. H. F. Judkins
Dr. C. W. Larson
Roberts Everett

1937

L. B. Esmond, Ch., Northville Laboratories
G. E. Putnam
Loomis Burrell
G. F. Kroha, The Pfaudler Co.
T. J. Newbill, National Dairy & Food Bureau
Jarvis Williams, Jr., Standard Cap & Seal Corporation
Dr. N. W. Hepburn
Dr. C. I. Corbin
Prof. H. F. Judkins
Roberts Everett

1938

Dr. L. A. Rogers, Ch., Chief, Division of Dairy Research Labs., Bureau of Dairy Industry, U. S. Dept. Agr., Washington, D. C.
Dr. C. I. Corbin
Dr. N. W. Hepburn
Prof. H. F. Judkins
L. B. Esmond
Henry Rosenstein, Thomas W. Dunn Company
Norman Thomas, Sr., Joe Lowe Corporation
Roberts Everett

WINNERS OF FELLOWSHIPS

The colleges winning the industrial fellowships, the names of the Fellows, and the institutions to which they were assigned for research are presented in Table 4.

TABLE 4

Colleges winning Dairy Industries Supply Association Fellowships and recipients of the awards 1930–1938 inclusive

No.	College	Name of Fellow	Year
1	University of Arkansas	Niven, Charles F.	1934
2	Connecticut State College	Gibson, G. L.	1932
		Kosikowsky, Frank V.	1938
3	Cornell University	Tomlinson, Albert S.	1936
		Brereton, John G.	1937
		Ludington, Varnum D.	1938
4	University of Illinois	Ross, O. E.	1930
		Corbett, W. J.	1933
5	Iowa State College	Ause, D. H.	1931
		Brown, Howard W.	1934
		Ford, Mark	1935
		Wilson, Charles A.	1938
6	Kansas State College	Germann, Ralph F.	1930
		Hostetler, P.	1933
		Chilson, H.	1933
		Byers, E. L.	1934
7	Massachusetts State College	Shepard, Sidney	1932
		MacCurdy, Robert D.	1937
8	Michigan State College	Dowd, L. R.	1930
		Haradine, C. E.	1931
		Babel, Fred J.	1934
		Openlander, H. F.	1936
9	University of Minnesota	Sorensen, C. M.	1933
		Josephson, Donald	1934
		Freeman, Robert	1935
		Rivers, Philip	1936
		Naylor, Brooks	1937
10	Mississippi State College	Quinn, J. D.	1930
		Whitfield, B. H.	1931
		Long, John H.	1932
		Carithers, R. L.	1933
		White, H. W.	1935
		Harris, William C.	1937
11	University of Nebraska	Kelley, Martin F.	1930
		Meredith, P. W.	1932
		Warner, James	1935
		Larson, Richard A.	1936
12	Ohio State University	Brockschmidt, J. H.	1931
		Homberger, R. E.	1931
		Charles, Donald A.	1932
		Slatter, Walter L.	1934
		Smith, Hiram P.	1935
		Adams, Joseph	1936
		Roahen, Dan C.	1937
13	Ontario Agricultural College	Goodwillie, D. B.	1930
14	Purdue University	Cantley, R. W.	1932
15	South Dakota State College	Spicer, Delmar	1934
		Henry, Dave	1937
16	University of Tennessee	Flake, J. C.	1935
		Robert, M. William	1936
17	University of Vermont	Leech, H.	1931

Here it will be noted that of the 17 colleges winning one or more fellowships, Ohio leads the list with seven, closely followed by Mississippi with six, and Minnesota with five. Iowa, Kansas, Michigan and Nebraska have each won four fellowships. It should be mentioned here that some teams and individuals from several schools, including those above mentioned, had sufficient high standing in judging to merit consideration, but were not qualified. Hence, in Table 4 are listed only the schools which, and individuals who, were finally awarded the grants.

ASSIGNMENT OF FELLOWS

Choice of schools in which to pursue graduate work was made in the order of the respective standings of the winning teams, or individuals, Table 5. No two Fellows were permitted to pursue graduate work at the same institution the same year; nor could a Fellow continue work at the same institution from which he graduated. Consequently, the research under these grants has been carried out in a wide selection of colleges, a

TABLE 5

Winners of Dairy Industries Supply Association Fellowships and the colleges to which they were assigned for research

Name	From	Individual* placing	Assigned to
	1930		
1. Ross, O. E.	Illinois	1st	Iowa
2. Goodwillie, D. B.	Ontario	2nd	Michigan
3. Quinn, J. D.	Mississippi	3rd	Ohio
4. Germann, Ralph F.	Kansas	4th	Pennsylvania
5. Dowd, L. R.	Michigan	6th	Purdue
6. Kelley, Martin F.	Nebraska	8th	Wisconsin
	1931		
1. Ause, D. H.	Iowa	1st	Minnesota
2. Homberger, R. E.	Ohio	3rd	California
3. Haradine, C. E.	Michigan	4th	Illinois
4. Whitfield, B. H.	Mississippi	5th	Nebraska
5. Leach, H.	Vermont	9th	Kansas
6. Brockschmidt, J. H.	Ohio	10th	Massachusetts
	1932		
1. Long, John H.	Mississippi	1st	Michigan
2. Meredith, P. W.	Nebraska	2nd	Ohio
3. Cantley, R. W.	Purdue	3rd	Cornell
4. Gibson, G. L.	Connecticut	5th	Illinois
5. Shepard, Sidney	Massachusetts	10th	Iowa
6. Charles, Donald A.	Ohio	11th	Wisconsin
	1933		
1. Corbett, W. J.	Illinois	1st	Wisconsin
2. Hostetler, P.	Kansas	2nd	Iowa
3. Chilson, H.	Kansas	3rd	Cornell
4. Carithers, R. L.	Mississippi	4th	Minnesota
5. Sorensen, C. M.	Minnesota	5th	Purdue
6. Spicer, Delmar	South Dakota	6th	Ohio

TABLE 5 (Continued)

Name	From	Team* placing	Assigned to
	1934		
1. Slatter, Walter L.	Ohio	1st	Iowa
2. Josephson, Donald	Minnesota	2nd	Pennsylvania
3. Byers, E. L.	Kansas	3rd	Wisconsin
4. Babel, Fred J.	Michigan	4th	Purdue
5. Brown, Howard W.	Iowa	5th	Illinois
6. Niven, Charles F.	Arkansas	6th	Cornell
	1935		
1. White, H. W.	Mississippi	1st	Ohio
2. Flake, J. C.	Tennessee	3rd	Purdue
3. Smith, Hiram P.	Ohio	4th	Illinois
4. Ford, Mark	Iowa	5th	Cornell
5. Freeman, Robert	Minnesota	6th	Connecticut
6. Warner, James N.	Nebraska	7th	Iowa
	1936		
1. Adams, Joseph	Ohio	1st	Purdue
2. Tomlinson, Albert	Cornell	3rd	Wisconsin
3. Larson, Richard	Nebraska	4th	Michigan
4. Rivers, Philip	Minnesota	5th	Pennsylvania
5. Roberts, M. William	Tennessee	6th	Minnesota
6. Openlander, H. F.	Michigan	7th	Ohio
	1937		
1. Roahen, Dan C.	Ohio	1st	Wisconsin
2. Harris, William C.	Mississippi	2nd	Iowa
3. MacCurdy, Robert D.	Massachusetts	3rd	Michigan
4. Naylor, Brooks	Minnesota	5th	Cornell
5. Brereton, John G.	Cornell	6th	Minnesota
6. Henry, Dave	S. Dakota	7th	Ohio
	1938		
1. Ludington, Varnum D.	Cornell	1st	(To be assigned)
2. Wilson, Charles Ashley	Iowa	2nd	
3. Kosikowsky, Frank V.	Connecticut	3rd	

* The fellowships were awarded from 1930 to 1933 inclusive, to eligible individuals on the basis of their proficiency in judging; since then, to members of the leading teams.

total of 14, as shown in Table 6. A total of 20 different schools have benefited one or more years by these grants. A number of schools have won a fellowship and have been assigned a Fellow from another institution the same year, thus two-fold benefits were received.

Three state colleges or universities, namely, Iowa, Ohio, and Wisconsin, have each been assigned six Fellows. Cornell and Purdue each have been assigned five, while Illinois, Michigan and Minnesota each have been assigned four. Pennsylvania has been assigned three, while California, Connecticut, Kansas, Massachusetts and Nebraska each have been assigned one Fellow. The three Dairy Industrial Fellows of 1938 remain to be assigned for research. Nine colleges or universities, Cornell, Iowa, Illinois, Michigan, Minnesota, Ohio, Pennsylvania, Purdue and Wisconsin, have thus directed 89.58 per cent of the research projects under the Dairy Industrial Fellowships.

TABLE 6

No.	College	Fellowships won by team or individuals†		Fellows assigned for graduate work‡	
		No.	*%*	*No.*	*%*
1.	University of Arkansas	1	1.96	0	0.00
2.	University of California	0	0.00	1	2.08
3.	Connecticut State	2	3.92	1	2.08
4.	Cornell University	3	5.88	5	10.42
5.	Iowa State	4	7.84	6	12.50
6.	University of Illinois	2	3.92	4	8.33
7.	Kansas State	4	7.84	1	2.08
8.	Massachusetts State	2	3.92	1	2.08
9.	Michigan State	4	7.84	4	8.33
10.	University of Minnesota	5	9.80	4	8.33
11.	Mississippi State	6	11.76	0	0.00
12.	University of Nebraska	4	7.84	1	2.08
13.	Ohio State University	7	13.73	6	12.50
14.	Ontario Agricultural	1	1.96	0	0.00
15.	Pennsylvania State	0	0.00	3	6.25
16.	Purdue University	1	1.96	5	10.42
17.	South Dakota State	2	3.92	0	0.00
18.	University of Tennessee	2	3.92	0	0.00
19.	University of Vermont	1	1.96	0	0.00
20.	University of Wisconsin	0	0.00	6	12.50
	Total fellowships	51	99.97	48	99.98
	Total schools	17		14	

† Fellowships were awarded to eligible individuals 1930–1933 inclusive, then to ranking teams.

‡ Fellows 1938 to be assigned.

PROBLEM ASSIGNMENT

To classify the research problems assigned and undertaken by the Industrial Fellows has been difficult because of the very nature of some of the problems. In many cases the problem applied to several products. An attempt was made, however, to classify them on the basis of some dairy product, realizing that other classifications, such as tests, methods of manufacturing, distribution, bacteriology and so on might have been used. The distribution is shown in Table 7. According to the classification employed, the research undertaken has dealt with some phase of butter, buttermilk, cheese, condensed milk, milk or ice cream. Thirty-one and three-tenths per cent of the projects have been closely associated with milk; 31.2 with ice cream; 16.6 with butter; 10.4 with cheese; and 4.2 per cent each with buttermilk and with condensed milk. The specific title of the project problems may be noted from the list of publications in Table 8.

PUBLICATIONS

The manuscripts, summarizing the results of one year's research on the specific problems, have been published entire or in part in one of several trade or scientific journals. These are listed in Table 8 by years, giving the names of the author or authors, title and where published. Recently a plan

TABLE 7

Distribution of research problems of association Fellows by years, 1930 to 1937 inclusive

Product*	1930	1931	1932	1933	1934	1935	1936	1937	Total	%
Butter		1	1		1	1	2	2	8	16.6
Buttermilk	1					1			2	4.2
Cheese	1			2	1			1	5	10.4
Condensed milk		1	1						2	4.2
Milk	2	2	3	3	1	2	1	2	16	33.3
Ice cream	2	2	1	1	3	2	3	1	15	31.2
Total	6	6	6	6	6	6	6	6	48	99.9

* Some projects may have applied to one or more products, for example, (1) the use of dry milk of different manufacture in ice cream or (2) the resazurin test in determining the quality of milk and ice cream. For the sake of clarity the problem was tabulated as far as possible with the product chiefly concerned in the research.

has been effected whereby the article may first be submitted for publication to a scientific journal, afterwards rewritten in popularized form for trade journal publication. The manuscripts resulting from the first five years of study have been published in the trade journals. The abstracting service of the various scientific journals has made available much of this material to the research field.

TABLE 8

Publications and research projects of the Dairy Industries Supply Association Fellows, by years, 1930 to present date

1930

1. Ross O. E. Why Does a Tallowy Flavor Develop in Strawberry Ice Cream? Ice Cream Trade Journal, July, 1933.
2. Goodwillie, D. B., and Trout, G. M. Factors Other than Bacteria That Influence the Body and Flavor of Granuled Buttermilk. The Milk Dealer, February and March, 1933.
3. Quinn, J. D., and Burgwald, L. H. High Short Holding and Low Long Holding Pasteurization of Milk. Milk Plant Monthly, February, 1933.
4. Bradley,* H. H., and Dahle, C. D. How Freezing and Hardening Affects the Texture of Ice Cream. The Ice Cream Trade Journal, November, 1933.
   * Completed work begun by Ralph Germann, Fellow, 1930.
5. Dowd, L. R. Some Factors Affecting the Efficiency of Pasteurization of Milk. American Creamery and Poultry Produce Review, January, 1935.
6. Kelly, Martin F., and Price, Walter V. A Study of the Manufacture of Cottage Cheese. National Butter and Cheese Journal, February, 1933.

1931

1. Ause, O. H., and Macy, H. The Relation of Oospora Lactis to the Keeping Quality of Butter. American Creamery and Poultry Produce Review, December, 1934.
2. Homberger, R. E., and Cole, W. C. Factors Affecting Lactose Crystallization as Related to Sandy Ice Cream. The Ice Cream Review, November, 1933.
3. Haradine, C. E. Inversion of Sucrose in the Manufacture of Sweetened Condensed Milk, and its Effect upon Color of Finished Product. National Butter and Cheese Journal, October, 1933.
4. Whitfield, Benjamin H., Davis, H. P., and Downs, P. A. The Effect of Milk upon Metals and Metals upon Milk. The Milk Dealer, November and December, 1934, and January, 1935.
5. Leach, H. J., and Martin, W. H. The Effect of a Surface Cooler on Flavor, Cream Line and Evaporation Loss. American Creamery and Poultry Produce Review, November, 1933.
6. Brockschmidt, J. H., Mack, M. J., and Frandsen, J. H. How to Make High Butterfat Ice Cream; A Study of the Factors Involved in Making the Richer Type of Product. Ice Cream Field, December, 1933, and January, 1934.

1. Long, W. John, Huffman, C. F., and Duncan, C. W. A Study of the Vitamin D Requirements of Calves When Natural Milk Furnished the Sole Source of the Antirachitic Factor. Milk Plant Monthly, July, 1936.
2. Meredith, Perry W., and Stoltz, R. B. Bottled Concentrated Milk, A Lower Priced Fresh Milk for the Consumer. The Milk Dealer, February, 1935.
3. Cantley, Robert W. Comparison of Skimmed Milk Powder Media with Standard Nutrient Agar for Bacterial Counts on Milk. Ice Cream Field, May and June, 1935.
4. Gibson, G. L. Sandiness: Its Causes and Prevention. Ice Cream Field, May and June, 1935.
5. Shepard, Sidney, and Olson, H. C. The Relationship Between Changes in the Number of Bacteria and in the Scores of Butter Held at 32° F. National Butter and Cheese Journal, September, 1935.
6. Charles, D. A., and Sommer, H. H. Causes and Practical Methods for Control of Sedimentation in Homogenized Milk. Milk Plant Monthly, April, 1935.

1933

1. Corbett, W. J., Frazier, W. J., and Price, W. V. A Gas Defect of Cream Cheese. The Milk Dealer, December, 1935.
2. Hostetler, Pius H. Effects of Preservatives on Results of Fat Test Studies. Confectionery and Ice Cream World, August, 1936.
3. Chilson, William H. A Study of the Oxidized Flavors of Market Milk. Milk Plant Monthly, November and December, 1935.
4. Carithers, Robert L., and Combs, W. B. Drum vs. Spray Process Dry Milk in Ice Cream. The Ice Cream Review, March, 1936.
5. Sorenson, C. M. Studies on Milk Mold Oospora Lactis. American Creamery and Poultry Produce Review, February, 1936.
6. Spicer, W. Delmar, and Burgwald, L. H. Use of Hydrogen Ion Determination on Young Cheese in Predicting Acid Development in Cheddar Cheese During Storage. National Butter and Cheese Journal, November, 1935.

1934

1. Slatter, Walter L. Changes in the Acetylmethylcarbinol Plus Diacetyl Content of Butter. National Butter and Cheese Journal, October and November, 1936.
2. Dahle, C. D., and Josephson, D. V. The Importance of the Fat Globule Membrane in the Freezing of Ice Cream. The Ice Cream Review, January, 1937.
3. Byers, E. L., and Price, Walter V. Influence of Salt on the Composition and Quality of Brick Cheese. National Butter and Cheese Journal, July, 1937.
4. Babel, F. J. Significance of Laboratory Tests in the Control of Ice Cream. The Ice Cream Trade Journal, September, 1936.
5. Brown, W. H. This Matter of Mix. Ice Cream Field, July and August, 1937.
6. Sherman, J. M., and Nivens, Charles F. The Hemolytic Streptococci of Milk. Journal of Infectious Diseases, March and April, 1938.

1935

1. White, H. W. Factors Affecting the Quality of Churned Buttermilk. Unpublished.
2. Flake, J. C. Some Causes for the Deterioration in 10 Days at 15.5° C. of Salted Butter Made from Sour Cream. Flake, J. C., and Parfitt, E. H. Journal of Dairy Science, September, 1938.
3. Smith, Hiram P. Variations in Color, Flavor, and Body Types of Ice Cream as Affecting Consumer Preference. Confectionery and Ice Cream World, February, 1938.
4. Knaysi, George, and Ford, Mark. A Method of Counting Viable Bacteria in Milk by Means of Microscope. Journal of Dairy Science, March, 1938.
5. Freeman, Robert. Sonic Vibration as a Means of Preparing Ice Cream Mixes. Unpublished.
6. Warner, James N. The Use of Resazurin in Determining the Sanitary Qualities of Milk and Ice Cream. Dairy World, February, 1938.

1936

1. Adams, Joseph, and Parfitt, E. H. Some Factors Influencing the Amount of Mold Mycelia in Butter. Journal of Dairy Science, May, 1939.

2. **Tomlinson, Albert S.** Enzymes in the Development of Oxidized Flavor in Milk. Unpublished.
3. **Larson, Richard A.** Relationship of Hydrometer Readings to the Composition and Some Physical Properties of Pan Condensed Ice Cream Mixes. Unpublished.
4. **Rivers, Philip.** Factors Affecting the Stability and Properties of Ice Cream Mix with Special Reference to Those Frozen in the Continuous Freezer. Unpublished.
5. **Roberts, William M.** Experiments with High Temperature Pasteurization of Cream for Buttermaking. Unpublished.
6. **Openlander, Herman.** The Use of Plain Frozen Condensed Milk in Ice Cream. Unpublished.

INFLUENCE OF GRADUATE TRAINING ON VOCATION OF FELLOWS

A study was made in the spring of 1938 of the vocations of the industrial Fellows and of the other team members and alternates to ascertain the influence of further dairy training upon the vocation of the student.

The percentage distribution of the vocations in which the team members, alternates and Fellows, from the 29 colleges competing in the national dairy products judging contest from 1930 to 1937 inclusive, were engaged at the time the study was made, is presented in Table 9. Inasmuch as six of the Fellows at that time were yet classified as undergraduate and that six other Fellows were engaged in research studies towards the master's degree, a total of 12, out of the total number of 48, it was difficult to classify their vocation other than as undergraduate and research. The percentage of Fellows graduated and engaged in the profession of their training is significantly higher, 45.8 per cent compared to 38.97 per cent, than those not having had the opportunity for further study. This high yield speaks well for the value of the Industrial Fellowships in furthering the training of men for the industry.

TABLE 9

Percentage distribution of all dairy products judging team members and alternates from 1930 to 1937, inclusive, according to their present occupations

Occupation	Association Fellows		All team members and alternates	
	No.	%	No.	%
1. Dairy plant (mgr., supt., res., lab., etc.)	22	45.8	191	38.97
2. Inspection and extension (federal, state, or city inspection, Co. Agt., 4-H Club agent, D.H.I.A., etc.)	2	4.2	54	11.02
3. Owner of plant (wholly or in part)	1	2.1	16	3.26
4. Teaching and research* or assisting (college and U.S.D.A.)	17	35.4	70	14.29
5. Undergraduate	6	12.5	57	11.63
6. Dairy farming	–		15	3.06
7. Vocational education, high school	–		21	4.28
8. Miscellaneous (unknown, insurance, automobile, etc.)	–		66	13.46
Total	48	100.0	490	99.97

* Including the six 1936 Fellows, who were engaged on their assigned problem when the data were compiled.

From this table it may be noted that approximately 39.0 per cent of all contestants and alternates, nearly 500, are plant owners, plant managers, superintendents, or commercial researchers. About 14.0 per cent are employed by colleges and the United States Department of Agriculture as teachers and researchers.

VALUE OF RESEARCH TO THE INDUSTRY

When the 1938 Dairy Industrial Fellows will have completed their graduate problem at the close of the academic year of 1939–1940, 51 selected men will have received graduate training in dairying and will have completed 51 dairy research projects. These men have profited personally from the opportunities afforded them through the Dairy Industrial Fellowships to continue their studies and to broaden their acquaintances. Likewise, the fund of scientific dairy information has materially increased while the concepts concerning dairy problems have been enlarged. Yet, the personal and specific benefits derived are insignificant in comparison to the inestimable value of the joint Dairy Industrial Research Program to the colleges, to the American Dairy Science Association, to the Dairy Industries Supply Association and to the dairy industry itself. Now in its ninth year, the program, as a part of the jointly sponsored students' National Dairy Products Judging Contest, has played a major role in dairy education, first, in furnishing incentives to the undergraduate and second, in making possible further dairy training to a selected group of dairy students.

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THE RIBOFLAVIN CONTENT OF COW'S MILK

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The riboflavin content of about 400 samples of milk produced in New York State has been determined. The presence of riboflavin in cow's milk is of considerable biological significance. Riboflavin is linked with biological oxidations (and possibly with the oxidations occurring in milk) in three ways: as the active group of an enzyme (12, 9), as a hydrogen acceptor (11), and as a photosensitizer for the oxidation of vitamin C (7, 4, 2).

The average riboflavin content of milk varies with the different breeds, the Jersey being highest, the Guernsey intermediate and the Holstein lowest. On the average, Holstein milk contains 33 per cent less riboflavin than Jersey milk. See Table 1. It is interesting that the order of breed is slightly different with respect to the carotene content of milk fat, in which case the Guernsey is higher than the Jersey.

TABLE 1

Riboflavin content of milk

Milk sample	Barn fed			Pasture fed		
	No. analyses	Mg. riboflavin per liter whole milk		No. analyses	Mg. riboflavin per liter whole milk	
		Range	Average		Range	Average
Jersey	25	1.55–3.42	2.18	24	1.90–3.40	2.37
Guernsey	14	1.64–2.72	1.91	14	1.41–2.52	2.04
Brown Swiss	4	1.80–2.08	1.87	5	2.00–2.62	2.37
Ayrshire	9	1.26–2.08	1.69	10	1.57–2.38	1.97
Holstein	22	0.60–2.31	1.42	23	1.19–2.44	1.64
Cornell Herd	74	0.60–3.42	1.80	76	1.19–3.40	2.04
Composite commercial	53	1.34–2.17	1.64	50	1.60–2.27	1.97

(Analyses on the Cornell herd were made on April 7 and June 8, 1937, and those on the composite commercial samples on April 4 and June 28, 1938.)

Commercial milk in summer was found to contain about 20 per cent more riboflavin than winter milk. This increase is apparently an effect of the diet and is true in general where the change in feed is from corn silage to pasture.

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The average increase in the riboflavin content of the milk of the Cornell herd in summer was 13 per cent. However the milk of cows receiving molasses-grass silage and phosphoric acid-grass silage was relatively high in winter and did not increase in riboflavin content when the cows were placed on grass pasture. Our observations confirm the work of Virtanen and Holmberg (10) who reported that with phosphoric acid silage the riboflavin content of the milk remained constant throughout the year.

A further study of seasonal and individual variation was made on 9 cows beginning April 13 and extending to June 29, 1938. A total of 164 analyses were made. Some of the cows were stall fed on molasses-grass silage and the rest were fed on phosphoric acid-grass silage and numerous samples were taken during the two weeks period preceding turning the cows out to grass pasture. There was no evidence of a change in riboflavin content when the cows were put out to graze. There was, however, considerable fluctuation with all the cows throughout the 10 weeks period. The maximum deviations of the 9 cows from their individual means were between ± 15 per cent and ± 27 per cent. The average value of riboflavin for all analyses for the 2 weeks before going on grass pasture feed was 2.28 and for the 2 weeks after going on grass pasture was 2.12, indicating no increase. Since the average deviation in all duplicate analyses was ± 3.99 per cent, any fluctuation less than this would be questionable with our method. During this period of 10 weeks the maximum deviation of the control solution of riboflavin from its mean value was ± 6.0 per cent. The change in feed produced practically no change in milk production which averaged 33.3 pounds per day before and 32.5 after the cows were put out on pasture.

The riboflavin content of milk varies with the individual cow, as well as with feed, breed and milk production. Variation among individual cows in a given season can be more than 500 per cent. The highest single milk sample observed on grass pasture contained 3.42 mg. per liter and the lowest contained 0.60 mg. per liter. Variations in individual, commercial, market milk samples were much smaller and the range was narrower, being from 1.60 to 2.27 mg. per liter. This was to be expected because the commercial samples were from mixed milk coming from various dairies throughout the state of New York. The samples were collected from delivery wagons.

Because of the variations due to breed, feed and individual cows, it is difficult to assign a single value as being the normal riboflavin content of milk. In place of a value, Table 1 showing group averages and Figure 1 showing distribution curves for milk samples with respect to riboflavin content are given. Individual variations in samples which are encountered in most biological work produce a need for a special type of analytical procedure. The methods used must be rapid and simple but need not be extremely accurate. It is much more significant to have a large number of analyses with an accuracy of ± 5 per cent than to have a few extremely accurate results.

The most frequently observed riboflavin content of the milk samples studied by us was about two mg. per liter. Our results are about 25 per cent higher than those of Whitnah, Kunerth, and Kramer (13). In our herd the few Ayrshire cows averaged higher than the Holstein cows, but the number of Ayrshire cows tested by us (10), is too small to establish this breed difference. Earlier analyses of the riboflavin in milk in individual samples are not inconsistent with our results. For example, Kuhn, Wagner-Jauregg, and Kaltschmitt (5) found 1.00 mg. riboflavin per liter of milk and von Euler, Adler, and Schlötzer (1) found 2 to 3 mg. per liter. Riboflavin in milk is reported to be 90 per cent free of combination with protein (5) and unesterified (6).

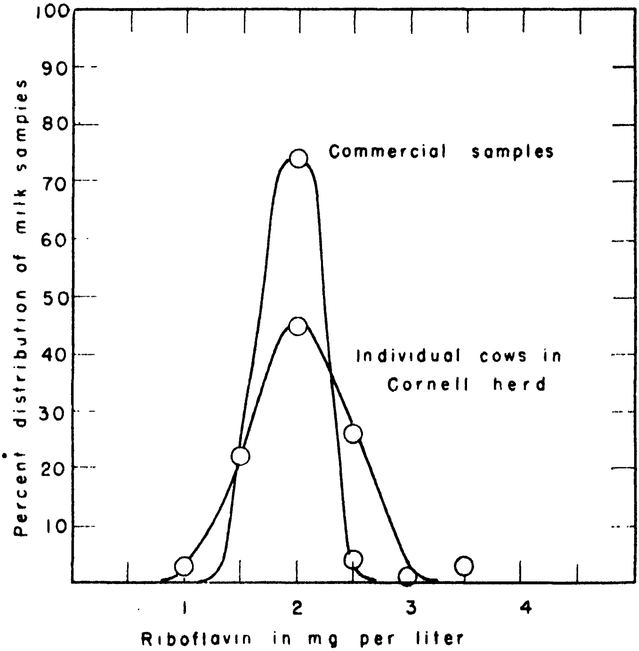

FIG. 1. Distribution of riboflavin in milk.

The determinations of riboflavin reported here were made by measuring the intensity of fluorescence of an acetone milk filtrate with a photoelectric cell and comparing this reading with the fluorescence of uranium glass as a standard (3). In order to make sure that variations were not due to fluctuations in the instrument, comparative readings were frequently made using a standard riboflavin solution which remained unchanged during the year.

No relation between riboflavin content of the milk and the development of oxidized flavor in the dark was observed with the samples studied. A definite inverse relation was found between riboflavin content and milk production, the linear correlation coefficient of which was -0.501 (± 0.058). This correlation was obtained with 76 cows, 5 different breeds, all receiving the same type of feed. The relation is even better than the linear correlation coefficient would indicate because the regression is not linear but skew. The data yield a correlation ratio of -0.830 (± 0.024). Since the square of the correlation ratio is 0.69 it may be concluded that two-thirds of the factors other than feed, which determine the content of riboflavin in milk may be accounted for by changes in milk production. A correlation of riboflavin with fat content has been reported (13) and it has been suggested that riboflavin is concerned in the production of milk fat. A correlation of riboflavin with fat content may result merely from the inverse correlation with milk production and thus offer no evidence that riboflavin is active in the production of milk fat. The evidence that there is little or no riboflavin in mare's milk (8) would indicate that riboflavin performs no function in the production of fat.

SUMMARY

The riboflavin content of about 400 samples of milk was determined by the measurement of the intensity of fluorescence of an acetone filtrate by a photoelectric cell. The riboflavin ranged from .60 to 3.42 mg. per liter. A negative skew correlation of -0.830 was found between riboflavin content and milk production. Commercial milk in summer contained about 20 per cent more riboflavin than winter milk. Cows, after being fed in winter on phosphoric acid grass silage, showed no increase in riboflavin when turned out on grass pasture.

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THE UTILIZATION OF SIMPLE NITROGENOUS COMPOUNDS SUCH AS UREA AND AMMONIUM BICARBONATE BY GROWING CALVES

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This problem has been studied, especially in Europe where protein concentrates are expensive. No attention, so far as we know, has been given to the problem in this country. The data from Europe are conflicting, some investigators claiming that polygastric animals can utilize for protein building simple nitrogen compounds like urea or ammonium bicarbonate, while others claim that they cannot. The foreign experiments have been balance experiments of short duration, which are always open to criticism, or experiments where urea, glycocoll or ammonium salts have displaced some of the protein for milk production. Here again the protein levels were sufficiently high so that the animals probably were receiving sufficient protein without a need for the nitrogen coming from simple forms like urea, glycocoll or ammonium salts. A complete review of this subject has been made recently by Krebs (1) and in this paper no further reference to the literature will be made. In this review by Krebs the conclusion was reached that the existing data on nitrogen balance experiments and protein displacement in lactation by urea or ammonium salts were inconclusive in respect to their utilization and that only long-time *growth* experiments could give conclusive data. This is in agreement with our own conclusions before inaugurating the experiments to be described.

Our experience with mineral balance experiments involving calcium and phosphorus, for example, has led us to the conclusion that erroneous interpretations from short-period experiments may at times be made. The best type of experiment for a study of the utilization of urea or ammonium nitrogen is one of long duration involving growth, milk production, etc.

FIRST EXPERIMENT

In our first experiment 4 calves, males, were started in the fall of 1936 upon the following ration:

Yellow corn	29.5	lbs.
Starch	20	"
Ground timothy hay	47	"

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Steamed bone meal	2 "
Iodized salt	1 "
Cod liver oil	0.5 "

This was to serve as the basal ration low in protein (Animal No. 1). It furnished a little less than 6 per cent of total protein (N × 6.25) and an intake of 8½ pounds of the ration daily contributed but 0.4 to 0.5 pound of total protein. Animal No. 2 received the basal ration plus urea in amounts so that the ration contained 18 per cent of protein (N × 6.25). Animal No. 3 received the basal ration supplemented with ammonium bicarbonate to a level where the ration contained 18 per cent of protein (N × 6.25); and the fourth animal the basal ration plus casein as a complex protein in such an amount that the ration contained 18 per cent of total protein (N × 6.25). It was planned to equalize the intake to the lowest individual consumption. The supplements were added at the expense of the combined quantities of corn, starch, and hay portions of the ration.

These animals were started in calf barracks but in November transferred to the basement of the Dairy Barn where a more constant temperature was available. With these rations as planned, the urea calf was getting about 66 per cent of its nitrogen from urea; the ammonium bicarbonate animal the same percentage of nitrogen from ammonium bicarbonate, and the casein animal the same percentage of nitrogen from casein. It took some time to work the animals onto the ration, the urea calf showing some diuresis—an excessive stimulation of the kidneys and excessive urination. After the calves were well on the ration, blood analyses were made to determine the level of total and serum protein, urea, and non-protein nitrogen. The total protein of the blood ran as follows:

Control animal	16.67%
Urea "	15.58%
Ammonium bicarbonate animal	14.20%
Casein animal	14.20%

The urea nitrogen distribution of the blood as run March 12, 1937, showed in mg. per 100 cc. of whole blood:

Control animal	7.2 mg.
Urea "	19.7 "
Ammonium bicarbonate animal	17.2 "
Casein animal	17.2 "

It is evident from these figures that the control animal was metabolizing very much less nitrogen than the other three, and also that the urea animal was carrying a slightly higher urea level in its blood than the ammonium bicarbonate or casein animal.

Growth records were made weekly. In February, in order to stimulate the appetites, 10 pounds of corn molasses—equivalent to 7 pounds of sugar—

were added to each 100 pounds of ration. This corn molasses was free from nitrogen. This addition had a very desirable effect in stimulating the appetites. Thereafter consumption equalization was very easy to make. From time to time the rations were increased from 8 pounds daily until at the end of the experiment each animal was receiving 12.5 pounds of the ration daily. Three charts showing the gains in weight over 28 weeks, and the 14-week period after the molasses was added as well as for the entire 40 weeks of the experiment, are shown. See Figures 1, 2 and 3. The experiment was run for 40 weeks during which time the control animal gained 201 pounds, the urea animal 290 pounds, the ammonium bicarbonate animal 276 pounds, and the casein animal 427 pounds.

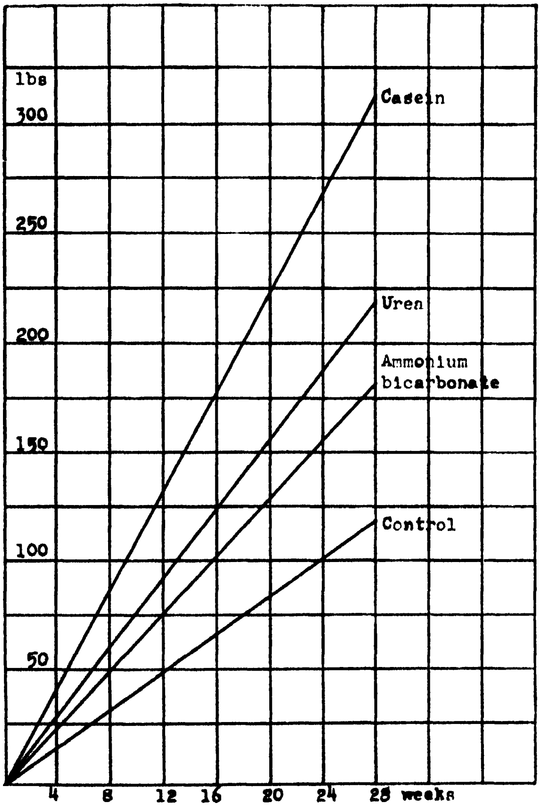

FIG. 1. Growth of male calves—28 weeks. Simple N compounds.

It is very apparent that these animals were making use of the simple nitrogen compounds. They were not utilizing them to the same extent as the protein casein, but nevertheless the growth rate of the urea and ammonium bicarbonate fed animals at certain periods approached that of the casein animal and all three were well ahead of the control. Apparently the control animal received sufficient protein to make slow growth although the protein was barely sufficient for what the standards specify for maintenance. During the 14 weeks recorded in Figure 2, the control animal gained about 65 pounds, the ammonium bicarbonate animal 105, the urea animal 110, and the casein animal 126 pounds—a little over a pound a day for all individuals receiving the extra amount of nitrogen. We continued the experiment until the animals reached 600 to 700 pounds and then slaughtered them for determination of the amount of stored protein in the carcass. This gave more positive data as to the utilization of these simple forms of nitrogen. Only with great difficulty could the entire carcass have been analyzed, but in lieu thereof slices were taken from the leg, shoulder, and rib, and in addition the liver, for determinations of moisture, fat, and protein. After drying, the analysis showed that these cuts were very much alike in composition. For

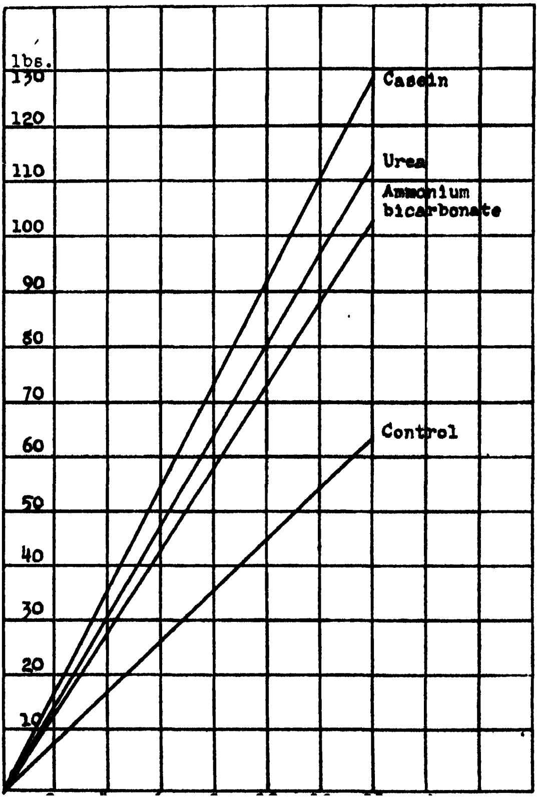

FIG. 2. Growth of male calves on simple nitrogen compounds. Molasses period. Equalized feeding.

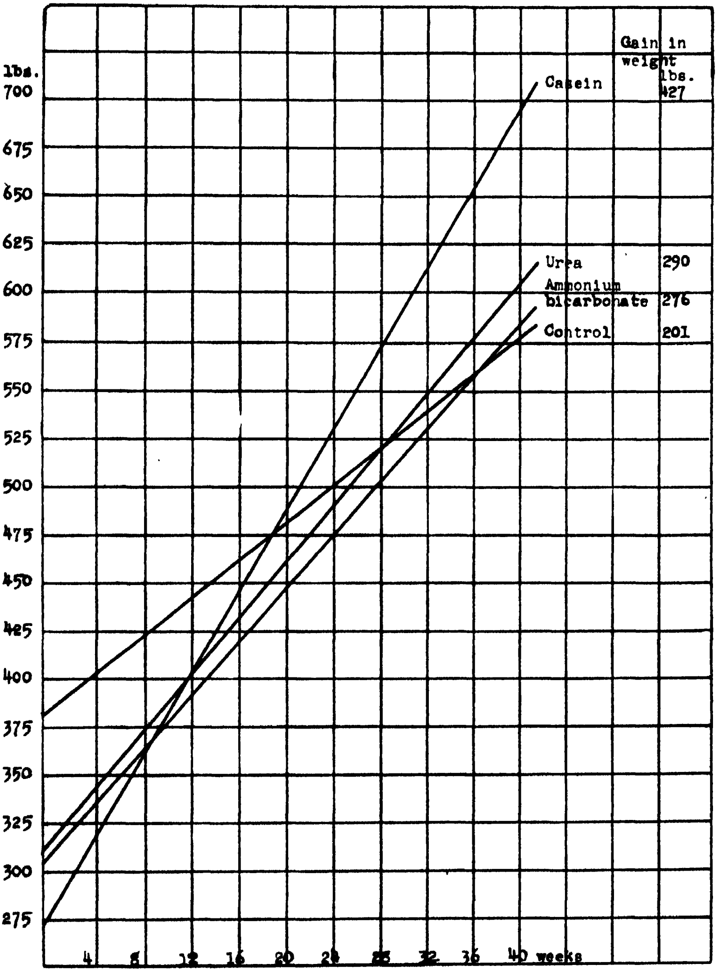

FIG. 3. Growth of male calves over period of 40 weeks.

details of this analysis see Table 1. As an example, the slice from the leg of the basal animal contained 76.95 per cent of protein (dry basis), urea animal 76.45, ammonium bicarbonate animal 80.49, casein animal 79.88.

These results would indicate that the increment in weight was of a constant composition and that the amount of protein in this increment was just as high on the very slow growth producing basal ration as on the ammonium bicarbonate ration, and that the protein in the growth increment of the ammonium bicarbonate and urea animals was just as high as in the casein animal. One can be fairly sure then that in this instance the growth record can be taken as proof of the utilization or partial utilization of ammonia and

TABLE 1

Experiment 1. Composition of tissues dried at 98° C.

	Protein content (N × 6.25)			
	Basal	Urea	NH_4	Casein
	%	%	%	%
Leg cut	76.9	76.4	80.4	79.8
Shoulder cut	71.4	65.3	71.3	65.2
Rib cut	65.8	72.4	69.4	65.5
Liver	59.8	63.5	70.3	62.0
	Fat			
Leg cut	15.6	14.2	10.5	13.6
Shoulder cut	24.6	25.5	22.2	29.5
Rib cut	28.6	22.5	21.9	29.0
Liver	11.3	9.8	11.4	11.3

urea through the intervention of bacteria in the rumen for the manufacture of protein. If the increment in weight is taken and the amount of protein determined in it over the entire period of the experiment, the data show that the control animal stored 35 pounds of protein, the urea animal 61.5, the ammonium bicarbonate animal 55, and the casein animal 86 pounds. It is evident that there was not as complete utilization of either the urea or ammonium bicarbonate nitrogen at the level fed as in the case of the casein. What level of urea or ammonium bicarbonate intake would serve best for maximum utilization must be determined by additional experiments.

SECOND EXPERIMENT

In the fall of 1937 another experiment was set up involving 6 Holstein heifer calves. The basal ration was modified to contain less protein by reduction of the corn in the ration, and corn molasses was included from the beginning of the experiment. The composition of the basal ration follows:

Yellow corn	20 lbs.
Ground timothy hay	47.5 "
Starch	24 "
Corn molasses	10 "
Special steamed bone meal	2 "
Salt	1 "
Cod liver oil	0.5 "

In the above ration 10 pounds of corn molasses, containing 25 per cent of water and free from nitrogen, was used. This ration contained 5.38 per cent of total protein (N × 6.25).

Calf 1 received the basal ration.

Calf 2 received the basal ration + 1.4 pounds urea per 100 pounds of ration. Total protein (N × 6.25) = 9.49 per cent. 43 per cent of nitrogen of ration from urea.

Calf 3 received the basal ration + 2.8 pounds urea per 100 pounds of ration. Total protein (N × 6.25) = 13.70 per cent. 61 per cent of nitrogen from urea.

Calf 4 received the basal ration + 4.3 pounds of urea per 100 pounds of ration. Total protein (N × 6.25) = 17.64 per cent. 70 per cent of nitrogen from urea.

Calf 5 received the basal ration + 11.1 pounds ammonium bicarbonate per 100 pounds of ration. Total protein (N × 6.25) = 17.04 per cent. 69 per cent of nitrogen from ammonium salt.

Calf 6 received the basal ration + 11 pounds of casein per 100 pounds of ration. Total protein (N × 6.25) = 15.52 per cent. 66 per cent of nitrogen from casein.

The supplements were made at the expense of the combined amounts of corn, starch, and hay. The calves were vigorous Holstein heifers and had an initial weight of 250 to 290 pounds. The heavier calf (290 pounds) was used for the basal ration and the 250 pound calf for the casein ration. Weather permitting they were turned out daily in a vegetation-free paddock. Equalized feeding was practiced throughout the entire experiment. Increments of intake were gradually made from week to week but all calves received equivalent allowances. The daily initial intake was 5.5 pounds and at the end of the experiment each animal was receiving 12.2 pounds daily. The appetites were good, but Number 1 on the basal ration was slow in complete consumption, sometimes requiring the entire day. On this basal ration no growth took place. The animal's coat became rough and shaggy, consumption was slow, the gait listless, and general condition poor. Constipation prevailed. There was no constipation in any of the other calves. Apparently the protein level was sufficiently low in this control ration to check growth but allow maintenance. There was no increment in weight for 12 weeks. In all other individuals, with the urea, ammonium bicarbonate, or casein supplements, definite growth was made from the initiation of the experiment. In the case of calves 3 and 4, where higher levels of urea were ingested, there was definite diuresis. In Table 2 are recorded the analyses of the blood of all individuals in respect to urea nitrogen, non-protein nitrogen, serum protein, and total protein. The blood samples were taken April 15, 1938 and one week before the control animal was given the basal ration supplemented with urea. It will be seen from the data that the serum protein was normal and constant in all animals. The control animal had not, at that time of analysis, shown a reduction in serum proteins. As was expected, the urea content of the blood varied, but in no case was it above the normal range. Total protein of the blood also was constant.

After 12 weeks on the basal ration, during which time there was no increase in weight, the ration of calf No. 1 was fortified with 1.4 pounds of urea per 100 pounds of feed. This made the composition of the ration identical with that fed calf No. 2. It was rather remarkable to note the change in this animal's condition; within 2 to 3 days the appetite quickened,

TABLE 2

Experiment 2. Blood composition

4/15/38

Animal	1	2	3	4	5	6
Urea N Mg./100 cc.	9.5	11.6	16.7	18.6	15.0	11.5
NPN Mg./100 cc.	27.9	31.9	39.2	43.4	34.1	30.9
Total protein per cent	17.6	18.35	16.95	17.49	17.45	17.40
Serum protein per cent	6.32	6.63	6.26	6.47	6.36	6.68

general apathy changed for the better and in the first week after the change in the ration there was an increase in weight of 11 pounds. Growth continued for 9 months at the rate of 1 pound per day, which was but slightly less than the growth rate of the casein fed calf (1.2 pounds per day) over a period of 10.5 months.

On June 28, 1938, the blood of all the calves was again analyzed for urea, non-protein nitrogen, serum protein, and total protein. These data are recorded in Table 3. Again the urea nitrogen was fairly constant, running at a slightly higher level in animal No. 4 which received the high urea ration. Total blood protein and serum protein were within the normal ranges. In Figure 4 can be seen the growth curves of all the animals over a period of 24 weeks. Number 1, after the change to the low urea level (1.4 pounds per 100 pounds of feed), No. 2, and the casein fed animal No. 6, grew quite alike and at times at a rate of 1 pound or more per day. This rate of growth is sub-normal, but for the type of ration used must be considered fairly good. Numbers 3, 4, and 5, all of which received the higher levels of urea or ammonium bicarbonate, did not make as rapid gains as the other 3 and averaged about 0.9 pound per day. Whether this was due to individual variation or to too high a concentration of the simple nitrogen compounds cannot be stated at present. Surely on the high levels of urea there was some waste of nitrogen as shown by the diuretic effect and the somewhat higher urea content of the blood.

TABLE 3

Experiment 2. Blood composition

6/28/38

Animal	1	2	3	4	5	6
Urea N Mg./100 cc.	14.1	10.6	13.2	16.4	11.3	12.8
NPN Mg./100 cc.	33.6	31.55	36.8	40.7	31.0	30.9
Total protein per cent	16.57	17.24	14.34	17.0	13.78	14.93
Serum protein per cent	6.61	7.05	5.77	6.56	5.77	6.31

When the animals had reached 592–678 pounds in weight, with gains of 307 to 427 pounds, and had been on these experimental rations for 12 months, they were slaughtered. As in the first experiment, cuts from the rib, shoulder, and leg were taken, and in addition the liver for determination of moisture, protein, fat and extractive non-coagulable nitrogen from the leg muscle. These data are displayed in Table 4.

The non-coagulable extractable nitrogen was made by taking 10 grams of the dry sample of leg muscle plus 50 cc. of water, heating to boiling for 4–5 minutes, cooling, and then filtering. The precipitate was washed twice with water and the nitrogen determined in this filtrate. It can be seen from the data in Table 4 that there is no indication that the urea or ammonium bicarbonate fed heifers contained more extractable nitrogen in the cut from the leg than did the casein fed animal. The blood data would also support the conclusion that, possibly with the exception of the animal receiving the high urea, there was no appreciable increase of urea in the blood, and consequently no holding by the tissues of soluble nitrogen. The data in Table 4 also show that the various cuts were quite alike in their protein content and that the urea-fed animals, as well as the ammonium bicarbonate-fed animal,

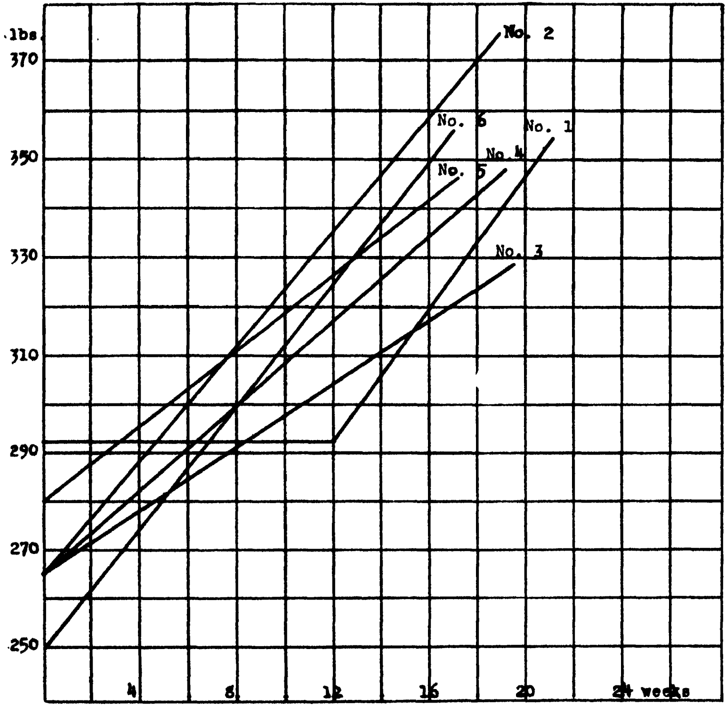

FIG. 4. Growth of heifer calves. Second experiment.
No. 1—Basal and basal + 1.4 lbs. urea.
" 2— " " 1.4 lbs. urea.
" 3— " " 2.8 " "
" 4— " " 4.3 " "
" 5— " " 11.1 " ammonium bicarbonate.
" 6— " " 11 " casein.

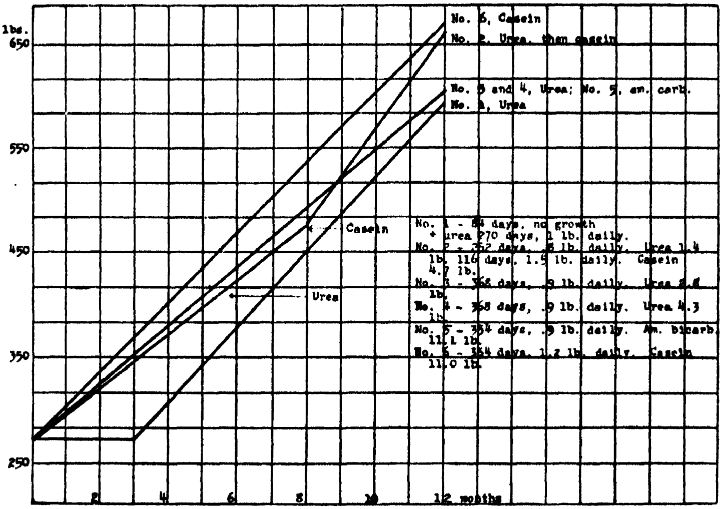

FIG. 5. Growth of heifer calves. Second experiment. Daily gain last 13 weeks:
No. 1—1.2 lbs.
" 2—1.4 "
" 3—1.2 "
" 4—1.0 "
" 5—1.0 "
" 6—1.4 "

TABLE 4'

Experiment 2. Composition of tissues (dried at 102° C.)

	Protein content (N × 6.25)					
	No. 1 Low urea	No. 2 Low urea then low casein	No. 3 Medium urea	No. 4 High urea	No. 5 Ammonium bicarbonate	No. 6 High casein
	%	%	%	%	%	%
Leg cut	84.6	86.2	89.4	85.9	85.5	85.3
Shoulder cut	84.8	87.3	87.9	87.0	83.7	83.8
Rib cut	54.4	57.3	68.3	58.2	58.2	54.8
Liver	62.1	62.8	66.3	65.1	64.7	64.3
Soluble non-coagulable protein (N × 6.25) Leg	8.0	7.5	6.9	7.6	6.9	7.2
	Fat content					
Leg cut	6.9	10.0	5.1	9.1	9.0	11.0
Shoulder cut	10.3	8.0	7.5	8.0	10.8	14.2
Rib cut	44.9	42.4	29.9	41.4	41.3	44.9
Liver	4.9	6.0	7.2	6.7	7.7	6.1

built tissue quite comparable in gross composition with the tissues built by the animal fed high casein.

At the time these animals were killed, food was withheld on the morning of the day of killing, which took place from 9 A.M. to 12 noon. Samples of rumen content lying next to the walls were taken for bacteriological examination as well as reaction. Those examined were No. 1 (low urea), No. 4 (high urea), and No. 6 (casein). The reaction of the material showed a pH of 7.8, 7.6, and 7.8 respectively. The bacterial cultures revealed no essential qualitative differences.[1] On each of the media used the material from the animal fed casein contained greater numbers of bacteria than did the other two. The numbers, however, were not significantly greater. The reaction of the rumen content was in agreement with that observed by Monroe and Perkins (2) in cattle fed timothy hay and grain, or timothy hay, grain, and corn silage. These investigators found that the reaction of the rumen content was about pH 7.5 before feeding and then the pH decreased to approximately 6.5 in 3–4 hours after feeding—after which it slowly rose again to 7.5. In their experiments it made no difference in the trend of the reaction whether the animal received hay and grain or hay, grain, and corn silage. Apparently the rumen content is subject to considerable mixing with the saliva, which may have a pH as high as 8.4.

As a matter of record it should be stated that all of these animals showed regular recurring estrous cycles.

At the time of killing the kidneys were removed and subjected to careful histological examination for evidence of pathological change. Grossly the only animal showing any hypertrophy of the kidney or evidence of kidney lesions was animal No. 4. This animal received the high urea level and showed considerable diuresis. Animal No. 1, which received 1.4 pounds of urea in 100 pounds of the ration, showed perfectly normal kidneys and no hypertrophy.

This amount of urea per 100 pounds of the total ration (1.4 pounds) is considerably above what would be recommended in practice for dairy cattle feeding, should the use of urea come into practice. On any low protein ration for dairy cattle, such as timothy hay, corn silage, and a home grown grain mixture of 50–50 corn and oats, not over 3 pounds of urea per 100 pounds of the grain mixture would be needed. Since the grain mixture would constitute about 1/3 of the total dry matter intake of the ration, urea would actually constitute about 1 pound in 100 pounds of the entire ration (dry matter basis). This is considerably less than the lowest level we used, namely, 1.4 pounds of urea per 100 pounds of ration. Consequently we would not anticipate, if urea feeding should come into practice and the levels indicated were used, that there would be any damage whatever to the kidney.

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[1] We wish to thank Professor Hastings and his assistants for making the bacteriological examinations and determining the reaction of the rumen content, and Dr. P. H. Phillips for the histological examination of the kidneys.

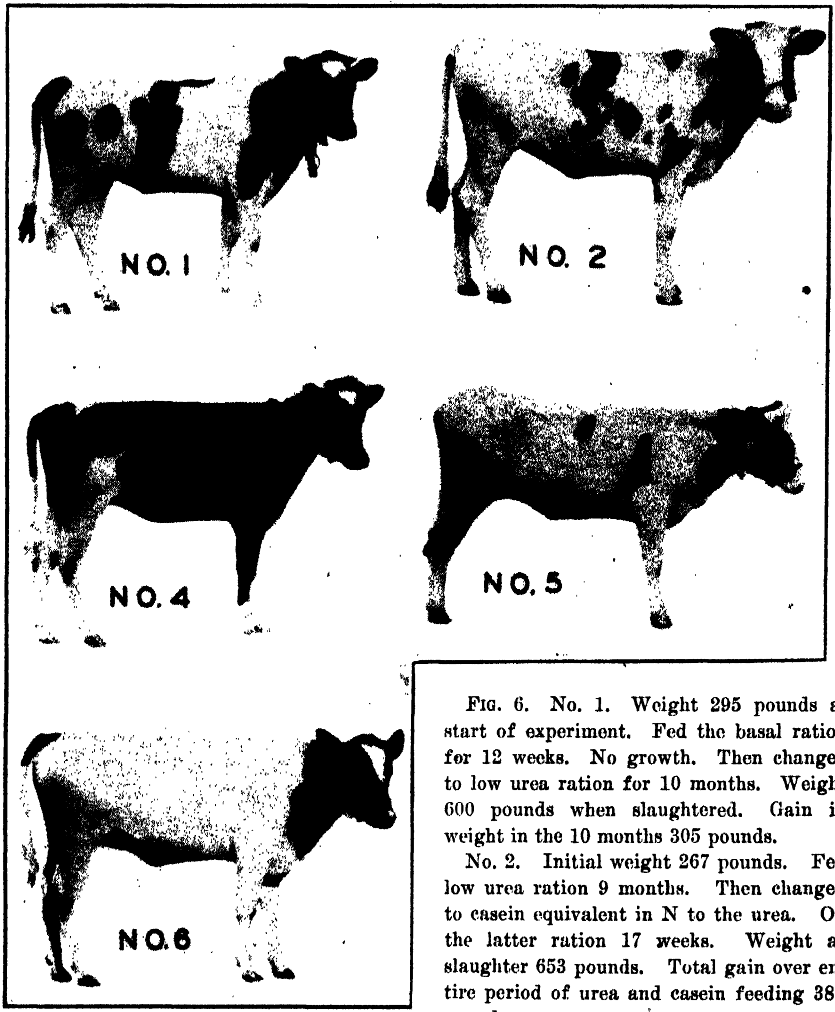

FIG. 6. No. 1. Weight 295 pounds at start of experiment. Fed the basal ration for 12 weeks. No growth. Then changed to low urea ration for 10 months. Weight 600 pounds when slaughtered. Gain in weight in the 10 months 305 pounds.

No. 2. Initial weight 267 pounds. Fed low urea ration 9 months. Then changed to casein equivalent in N to the urea. On the latter ration 17 weeks. Weight at slaughter 653 pounds. Total gain over entire period of urea and casein feeding 386 pounds.

No. 4. Initial weight 264 pounds. High urea ration. Weight 592 pounds at slaughter. Gain 328 pounds.

No. 5. Initial weight 273 pounds. Ammonium bicarbonate ration. Weight at slaughter 608 pounds. Gain 335 pounds.

No. 6. Initial weight 251 pounds. High casein ration. Weight at slaughter 678 pounds. Gain 427 pounds.

Detailed histological studies showed that No. 1 (low urea), No. 2 (low urea and low casein), No. 5 (ammonium bicarbonate), and No. 6 (high casein) had normal kidneys and the only evidence of histological disturbance was a slight congestion in the kidneys of all animals.

No. 3 (medium urea) showed such tissues, as the spleen, liver, and muscle, to be normal; but the kidney showed slight but definite evidence of damage.

No. 4 (high urea) showed the spleen and muscle as normal. In the liver there were areas of necrosis. The kidney was badly damaged with hyaline degeneration and many casts in the tubules.

DISCUSSION

The data from the two experiments indicate definitely that urea and ammonium bicarbonate nitrogen can be used by growing heifers when part of the protein comes from grains and timothy hay. In the case of urea feeding, high levels of intake led to diuresis and a slight increase of urea level in the blood. Apparently somewhat better utilization and little if any diuretic effect was obtained when the level of urea was 43 per cent of the nitrogen ingested as compared with the higher levels of 66 and 70 per cent. Further experiments must determine if lower levels of urea than those used in these experiments would be still more effective. Our experience with ammonium bicarbonate is limited to the two levels of 66 and 69 per cent of the nitrogen ingested. Here again better utilization may take place at lower concentrations. The ration containing ammonium bicarbonate lost some of its nitrogen on standing, the odor of ammonia being apparent. In one analytical run the total protein (N × 6.25) of the ration receded to 12.2 per cent from the initial theoretical amount of 17.0 per cent.

It is probable that ammonium bicarbonate would be less suitable as a simple form of nitrogen than urea. It is apparent from present experience that best utilization of these simple nitrogen compounds will take place when the ration contains some readily soluble sugar. This seemed to be the indication from our first experiment but further trials will be needed to determine whether this is always the case. Apparently the medium for bacterial multiplication would be improved by the ingestion of some soluble carbohydrate. The most logical theory for the utilization of these simple nitrogenous materials could be stated as follows: The bacteria of the rumen find the medium of simple nitrogenous salts and sugar an excellent one in which to grow. Through their multiplication they build proteins which would contain the amino acids necessary as supplements to the proteins of the ration. These bacterial cells pass from the rumen to the fourth stomach where they are digested and become just so much protein for the animal. This seems to be the simplest explanation at present. Apparently there must be some protein in the ration for effective utilization of urea. With lambs Morrison and associates[2] secured practically no storage of nitrogen where urea nitrogen constituted nearly all of the nitrogen of the ration. One would infer from their experiments that bacterial cell proteins formed in the rumen of the lamb were incomplete in respect to the essential amino acids.

It should be emphasized that utilization of simple forms of nitrogen would be expected only in polygastric animals such as sheep, goats, and cows, and not in pigs or chickens.

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[2] Private communication to Professor Bohstedt.

There are some practical aspects of the problem worth mentioning. First, in the making of molasses legume silage, some of the protein nitrogen is converted to ammonia. It is apparent that this degradation may not mean a complete or even a partial loss of nitrogen. Second, it is also apparent that fertilization of pastures with nitrogen salts, whereby the nitrogen of the plant is appreciably increased, would mean that this increased nitrogen could in all probability be utilized by this type of animal just as well as if it were finished protein. Third, it is entirely possible that the improvement of a low protein ration of poor biological value can be made through the use of molasses and urea or possibly through urea alone; that the proteins formed in these bacterial cells may supplement the rest of the ration better than some protein concentrates that are now in use. This is a problem for the future.

SUMMARY

1. Two separate experiments involving the utilization of urea and ammonium bicarbonate nitrogen with 10 growing calves have been conducted.

2. The experiments indicate definitely that such nitrogen can be used for at least a partial supply of protein nitrogen. The most probable explanation is the production of protein by bacterial cell multiplication in the rumen and later digestion in the fourth stomach and intestine. Apparently most efficient utilization occurs when some soluble sugar such as corn molasses is fed in the ration.

3. With urea nitrogen constituting 43 per cent of the nitrogen of the ration the growth rate was but slightly less than that secured with a ration containing 66 per cent of its nitrogen as casein nitrogen. This does not imply that urea nitrogen in the ration used is superior to casein nitrogen.

4. Where casein nitrogen constituted 43 per cent of the total nitrogen of the ration, as in the case of animal No. 2, the growth rate over a period of 16 weeks was 1.5 pounds daily as compared with 1.3 pounds daily for the comparable level of urea nitrogen.

5. The nitrogen of ammonium bicarbonate was also utilized.

6. Analysis of the tissues at the end of the experiment indicated that the increments in weight with urea or ammonium bicarbonate nitrogen were of normal composition with a protein content similar to that of the casein fed animal. Extractable non-coagulable nitrogen was no greater in the leg tissues of the urea or ammonium bicarbonate animal than in the tissues of the casein animal.

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THE INFLUENCE OF THE PHYSICAL CHARACTER OF THE ROUGHAGE ON THE PER CENT OF FAT SECRETED IN THE MILK[1]

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Recently it was reported: "the fat content of the milk produced may be varied more than sixty per cent, at will, for the entire lactation by changing the physical characteristics of the roughage which the cow receives. This can be done at will and without changing the quantity of milk produced."[2] Two trials at this Station, in which the roughage was ground, should throw additional light on this subject. In the first double-reversal trial, a grain mixture consisting of three parts ground corn, three parts ground oats and one part linseed oil meal was fed, together with corn silage and a good grade of alfalfa hay. During alternate periods the alfalfa was cut into lengths of one-fourth inch and fed mixed with the grain. Actually much of the alfalfa was reduced to a powder or was in the nature of a finely ground meal. Four animals in each group completed the trial. The results of this trial are shown in Figure 1.

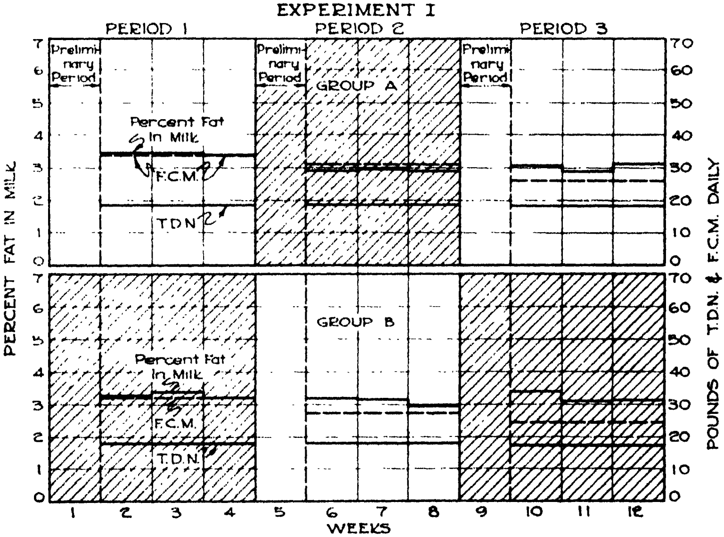

FIG. 1. Results obtained when uncut alfalfa was compared with fine cut, "ground," alfalfa for dairy cows. The four-week periods in which the roughage was uncut are shown by cross-hatching.

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[1] Journal Paper No. J-636 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 416.

[2] POWELL, E. B. One cause of fat variation in milk. Mimeographed abstract of paper presented at the annual meeting of the American Society of Animal Production, Chicago, December, 1938.

In the second double-reversal trial, a grain mixture consisting of two parts of ground corn, two parts ground oats and one part linseed oil meal was fed, together with corn silage, alfalfa hay and corn fodder. About seven and one-half pounds of uncut alfalfa was fed daily to each cow in each period of this experiment. During alternate periods approximately half of the silage was replaced with a mixture of 40 parts mow-cured alfalfa and 60 parts corn fodder, ground as in Experiment I. The amount of ground roughage fed varied between 10 and 15 pounds, depending on the cow. The daily intake of total digestible nutrients, the amount of fat corrected milk produced daily and the percentage of fat in the milk for each period are shown in Figure 2. In this trial, the data on five cows in group A and four cows in group B were available for the three periods.

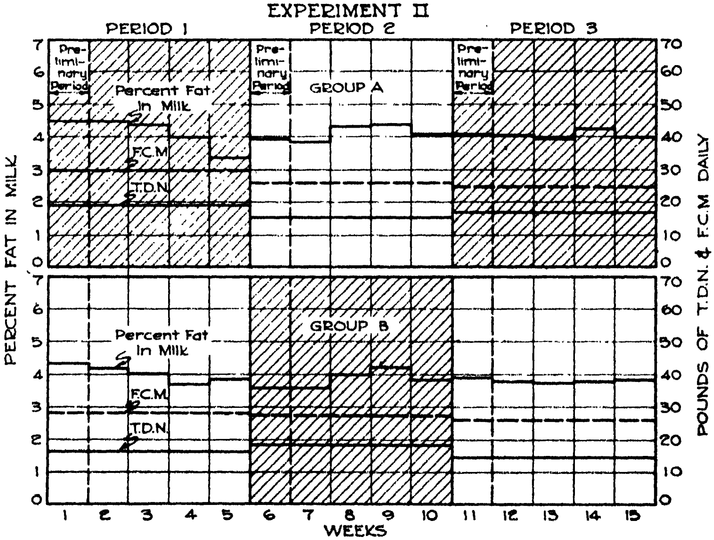

FIG. 2. Results obtained when part of the silage was replaced by a mixture of finely cut, "ground," alfalfa and corn fodder in the ration of dairy cows. The five-week periods in which the roughage was cut are shown by cross-hatching.

From the results shown in these two graphs it is evident that the percentage of fat in the milk was not materially influenced by grinding to a meal the dry roughage in the ration.

In the first experiment where ground alfalfa hay was fed, the average daily intake of total digestible nutrients was remarkably constant for the three periods. As would be expected with advancing lactation there was a slight drop in the amount of milk (in terms of four per cent milk) secreted. The per cent of fat in the milk failed to show any upward trend during the twelve weeks in which the trial was being conducted. Neither is any upward or downward trend indicated in the per cent of fat in the milk as a result of changing the physical character of the dry roughage fed.

Very similar results were obtained in Experiment II. Here the total digestible nutrient intake was slightly higher during the periods in which ground roughage was fed. However, the net energy available would not vary greatly. The amount of milk (in terms of four per cent milk) secreted was even more constant for the three periods than in Experiment I while the percentage of fat in the milk varied slightly. In this trial, the percentage of fat in the milk of both groups frequently moved in the same direction, even though they were on different rations, indicating that probably the environment and neither the stage of lactation nor type of feed caused the fluctuation in fat percentage.

In the work referred to in our introductory statement, no explanation was given why the per cent of fat in the milk should vary on changing the physical character of the roughage and we see no reason why the test should change under these circumstances. The results of trials at this Station which have been presented do not confirm the conclusion that changing the physical characteristics of the roughages will influence the fat percentage of milk. It is recognized that in these experiments, which were originally set up to study the cost of grinding roughage, no trials were run in which all of the roughage, both hay and silage, in the ration was ground. Had this been done, it might have given entirely different results.

CAROTENE INTAKE, LEVEL OF BLOOD PLASMA CAROTENE, AND THE DEVELOPMENT OF PAPILLARY EDEMA AND NYCTALOPIA IN CALVES*

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In a previous publication Moore, Huffman, and Duncan (1) summarized the literature and reported observations on 24 cases of a type of blindness in the bovine due to a constriction of the optic nerve where it passes through the bony optic canal. The evidence indicated that the blindness was of a nutritional origin. Moore (2) has recently presented evidence to show that the blindness was due to a deficiency of vitamin A and was associated with the development of nyctalopia and papillary edema. Nyctalopia is a condition of night blindness in which the individual fails to see properly in dim light, but can see in bright light. Papillary edema is a swelling or an edema of the nerve head in the eye. In order to diagnose the latter condition it is necessary to examine the nerve head with an ophthalmoscope. For further details and a discussion of these conditions the previous paper may be consulted (2). Cunningham and Addington (3) have recently published results showing the type of blindness due to constriction of the optic nerve in calves whose dams received a ration of ground Hegari fodder and cottonseed meal. McNutt and Wall (4) have also reported the same type of blindness in feeder steers in Iowa. It is the purpose of this paper to show the relationship between carotene intake and the level of plasma carotene on the development of nyctalopia and papillary edema in calves when the carotene intake was under control.

EXPERIMENTAL

Plasma carotene determinations were made each week according to a recently published method (5). For the carotene determinations of the alfalfa meal the extractions were carried out by the method of Guilbert as modified by Peterson, *et al.* (6) and determined by the use of a photoelectric colorimeter.

Male calves of the Holstein and Ayrshire breeds were used. They received a low carotene ration after 40–90 days of age which consisted of skimmed milk and a concentrate mixture consisting of 240 pounds barley, 180 pounds rolled oats, 180 pounds wheat bran, 60 pounds linseed oil meal and 8 pounds salt. Previous to being placed on the ration the calves received whole milk and the low carotene concentrate ration. The concentrate ration contained 0.5 to 0.7 microgram of carotene per gram so that each calf received 2 to 3 micrograms of carotene per pound of body weight from this source. Wood shavings were used as bedding.

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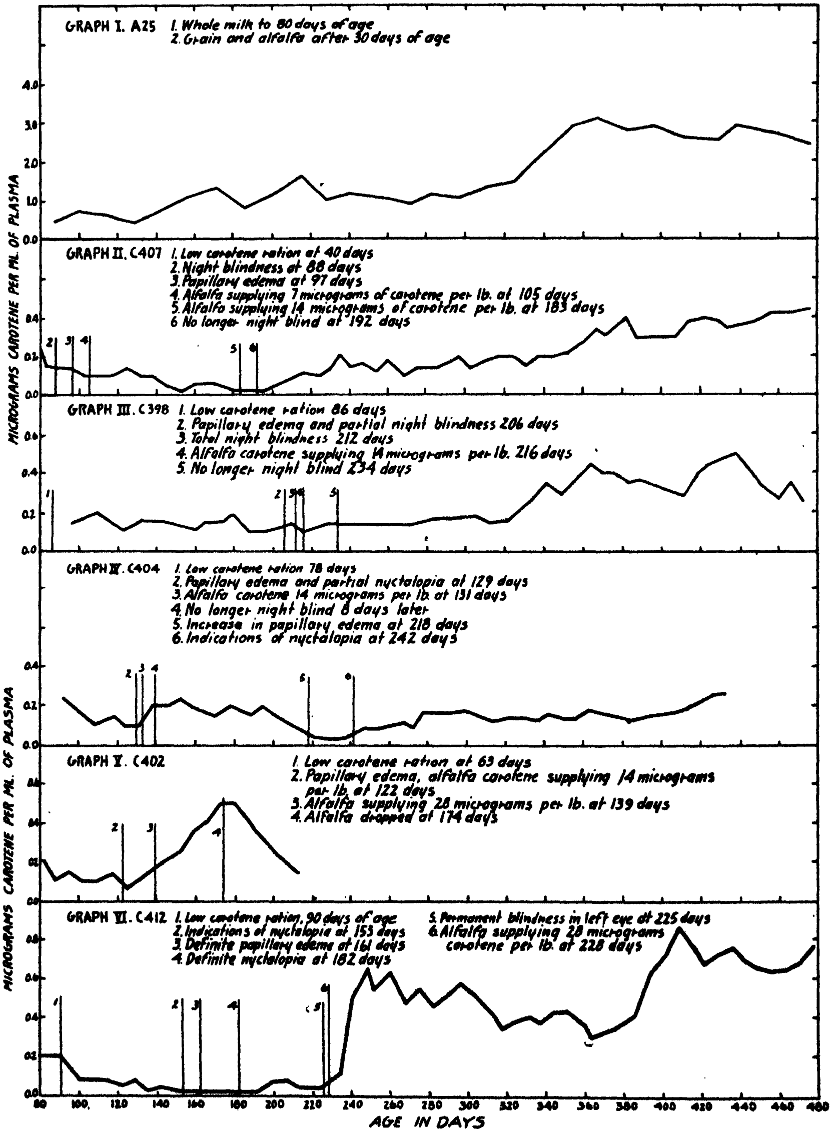
After the calves were placed on the low carotene ration, ophthalmoscopic examinations were made weekly. The calves were tested for night blindness each week by attempting to run them into objects and observing their behavior in dim light, a method similar to that used by Guilbert and Hart (7). The calves were weighed every 10 days at which time adjustments were made in the amounts of carotene fed.



In order to compare the level of blood plasma carotene of a normally fed calf with the experimental animals, graph I shows the plasma carotene content of calf A25. This calf received whole milk to 80 days of age, a concentrate ration and alfalfa in liberal quantities after 30 days of age. It will be noted that a different scale for plasma carotene was used in this graph than in the graphs for the experimental animals.

Calf C407, an Ayrshire, was placed on the low carotene ration at 40 days of age and the principal results are shown in graph II. This calf was myopic by 2 to 3 diopters at the beginning of the experiment. At 81 days of age there was some suspicion of nyctalopia which was definite at 88 days. At 97 days of age there was some definite swelling of the nerve head and the orange yellow color of the tapetum lucidum was bleached. By this time the plasma carotene had dropped to 0.1 microgram per ml. At 105 days of age alfalfa was added to the ration which supplied 7 micrograms of carotene per pound of body weight or approximately one-half of the Guilbert and Hart (7) requirements. This was in addition to that received in the ration. The edema of the nerve head continued to increase so that the margins were completely blurred. The calf coughed considerably and scoured. The plasma carotene continued at an extremely low level. During part of this period the calf was only partially night blind. The left eye showed exophthalmus and the capillaries of the retina showed considerable distension. At 183 days of age the calf was in such poor condition that it was necessary to increase the carotene intake to 14 micrograms per pound. Nine days later the calf was no longer nyctalopic but showed exophthalmos in both eyes. The animal showed gradual improvement in condition and the plasma carotene gradually increased. The edema of the nerve heads decreased considerably. However, this animal never regained his full activity nor physical appearance, and always moved about rather slowly. He probably came very near to developing the permanent type of blindness due to constriction of the optic nerve so that the visual acuity was possibly decreased some by post papillitic atrophy.

Calf C398, an Ayrshire, was placed on the low carotene ration at 86 days of age as shown in graph III. This animal had a congenital posterior lenticonus in both eyes making it difficult to examine the nerve head. At 206 days of age this animal showed nyctalopia and papillary edema. At 216 days when the swelling of the nerve heads had increased to 2 diopters, alfalfa was added to the ration supplying 14 micrograms of carotene per pound of body weight. Eighteen days later the calf was no longer night blind. The papillary edema receded very slowly and was still present after one year of supplemental feeding at the 14 microgram level. The plasma carotene increased gradually after the supplemental feeding.

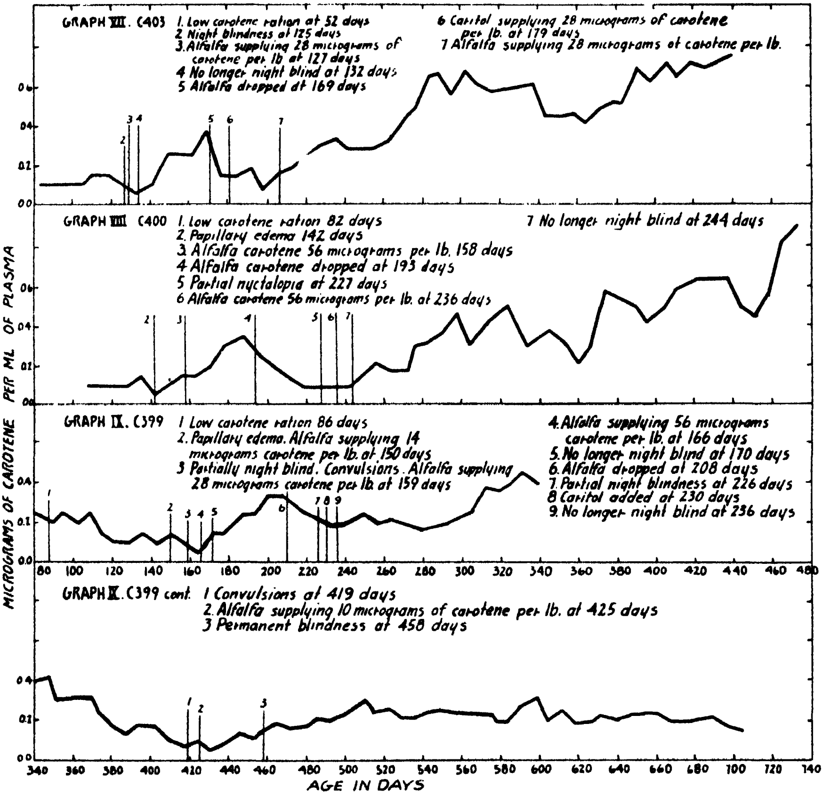
Calf C404, also an Ayrshire, was placed on the low carotene ration at 78 days of age as shown in graph IV. At 129 days there was definite edema of the nerve heads of both eyes, the right eye showed a choking of 1.5 D while the left eye showed a choking of one-half D. Partial night blindness was also observed and the tapetum lucidum of both eyes showed considerable bleaching. The plasma carotene had decreased to the 0.1 microgram level.

Alfalfa supplying 14 micrograms of carotene per pound of body weight was added at 131 days and after 8 days this calf was no longer night blind. The plasma carotene increased considerably and then decreased later for some unknown reason. At this time the nerve head of the right eye showed some increase in edema and at 242 days there were some indications of night blindness. At 435 days of age the condition of the eye ground showed little change from that noted at 218 days and although the tapetum lucidum took up some slight color it never assumed the orange yellow color which is normal for the structure. It will be noted that the plasma carotene remained quite low after the addition of the alfalfa and it was not until the latter stage of the experiment that the values rose above 0.2 microgram per ml.

Calf C402, a Holstein, was placed on the low carotene ration at 63 days of age, as illustrated in graph V, and showed evidence of papillary edema at 122 days of age at which time the plasma carotene had declined to a low level. Alfalfa supplying 14 micrograms per pound of body weight was added to the ration, and this allowance was increased later to 28 micrograms after which the plasma carotene increased from 0.04 to 0.5 microgram per ml. At 174 days the alfalfa was removed from the ration and the plasma carotene started to decline. At 213 days the calf died due to injuries not related to the experiment.

Calf C412, a Holstein, started to receive the low carotene ration at 90 days of age and at 161 days definite papillary edema developed as shown in graph VI. At 182 days definite nyctalopia was present. At 203 days the edema had increased considerably in the left eye so that there was a choking of 2 D. Many of the capillaries and small vessels at the edge of the nerve head also showed considerable fusiform dilation at this time. At 225 days the pupil of the left eye did not react to light stimulation and showed mydriasis. By means of covering each eye separately it was found that in daylight the calf was completely blind in the left eye but could still see well out of the right eye. However, in the dark the animal showed complete night blindness. Both eyes showed some exophthalmus. It will be noted that the plasma carotene had remained at an extremely low level during this period. At 228 days carotene was added to the ration in the form of alfalfa hay in an attempt to preserve the sight of the right eye. The attempt was successful for at the age of 480 days the calf could still see. The addition of the alfalfa promoted an immediate rise in the plasma carotene.

Calf C403, a Holstein, was placed on the low carotene ration at 52 days of age and night blindness developed at 125 days of age as shown by graph VII. Papillary edema was not present. At 127 days alfalfa supplying carotene at the rate of 28 micrograms was added. Five days later the night blindness had disappeared and the carotene content of the blood plasma increased from 0.03 to 0.34 microgram per ml. by 169 days at which time the alfalfa was discontinued. "Caritol" supplying 28 micrograms of carotene per pound was added at 179 days. However, the plasma carotene appeared to decrease further so that at 205 days the alfalfa was substituted after which there was a steady increase in the level of plasma carotene.



Calf C400, an Ayrshire, as shown in graph VIII, was placed on the low carotene ration at 82 days of age and at 142 days the right eye showed a choking of 1 D and considerable edema was present on the dorsal edge of the nerve head. The left eye showed no choking but the nerve head was somewhat hazy. By this time the plasma carotene level had dropped to .04 microgram per ml. At 148 days there was considerable hemorrhage on the retina of the left eye along the vertical vessel. The hemorrhage was 2 cm. in length and 1 cm. in width as seen through the ophthalmoscope. There was also some involvement of the anterior chamber. At this time there was no indication of night blindness. At 150 days alfalfa was added to supply 56 micrograms of carotene per pound of body weight. At 162 days both eyes showed a choking of 2 D and the nerve margins were entirely blurred. The hemorrhage in the left eye was present until 185 days although it gradually decreased in size during this period. At 193 days the feeding of alfalfa was discontinued whereupon the plasma carotene began to decrease. At 227 days it had dropped to 0.1 microgram per ml. and the calf showed nyctalopia. At 236 days carotene feeding was resumed at the previous rate and 8 days later evidence of night blindness had disappeared. The plasma carotene showed a gradual rise and attained values up to 0.6 microgram per ml. The edema of the nerve heads seemed to persist and the area previously covered by hemorrhage gradually assumed a greenish blue color. At 350 days the edema of the left eye had receded considerably and about two-thirds of the margins of the nerve head were discernible. The fibers of the nerve head could again be seen. At 415 days the edema in both eyes had largely disappeared and both eyes appeared to be quite normal except for the discolored area in the left eye due to previous hemorrhage.

Calf C399, a Holstein, was placed on the low carotene ration at 86 days of age as shown in graph IX and at 150 days showed evidence of papillary edema with a choking of 2 D in the left eye but was not night blind. At this time alfalfa was added supplying 14 micrograms of carotene per pound. At 159 days the calf showed evidence of nyctalopia and had a convulsion in the pen at the time of testing for night blindness. Because of the animal's apparent poor condition the allowance was further increased to 28 micrograms. At 164 days the right eye showed a choking of 1.5 D and the left eye 3 D. The development of a posterior polar cataract was observed in the right eye. At 166 days the alfalfa intake was increased so as to supply 56 micrograms of carotene per pound. The alfalfa was discontinued at 208 days and at 226 days nyctalopia was observed again. The plasma carotene had dropped to 0.2 microgram per ml. "Caritol" was added at 230 days and the calf was no longer night blind 6 days later. The "Caritol" was supposed to supply 56 micrograms of carotene per pound of body weight. The level of plasma carotene increased for a while and then decreased. The "Caritol" mixture apparently underwent auto-oxidation after a preliminary induction period so that no comparison could be made as to the effectiveness of this source of carotene.

The calf was in rather poor condition at 419 days, had a few convulsions and showed incoordination. The plasma carotene had decreased to the 0.05 microgram level, and at 425 days alfalfa supplying 10 micrograms of carotene per pound of body weight was added. During this period, observations on night blindness were not made because of the further development of the cataract in the right eye which would complicate the observations. At 458 days the calf went entirely blind so that the 10 microgram level did not prevent the further development of this condition. It will be noted that the plasma carotene level remained at about 0.2 microgram per ml. At this level of carotene intake the incoordination disappeared but the calf never seemed to pick up in physical appearance to any extent.

DISCUSSION

From the results obtained from this group of Holstein and Ayrshire calves it was found that when the level of plasma carotene fell to approximately 0.13 microgram per ml. or below, nyctalopia and papillary edema were likely to develop in a short while. Furthermore, if continued, the permanent type of blindness might also develop as in the case of calves C412 and C399. C412 remained at an extremely low level for over 3 months before the blindness developed. There are of course individual variations.

When the calves were placed on the low carotene ration at 40 to 90 days of age, nyctalopia developed in from 48 to 73 days while one animal, C398, went for 120 days which seemed to be the exception. The calves received whole milk as the only source of vitamin A up to the time they were placed on the low carotene ration. Papillary edema developed at about the same time although there were some variations. These variations are due to the fact that the two conditions are the result of two different processes as explained in a previous paper. It seems probable that with the method used only the later stages of night blindness were detected.

The nyctalopia could be cured in from 5 to 18 days by the addition of carotene to the ration.

From the results obtained from Calf C407, the addition of 7 micrograms of carotene per pound of body weight to the basal ration, which would make the total intake about 9 micrograms per pound, was not sufficient to raise the level of plasma carotene above 0.2 microgram per ml. This level did not prevent nyctalopia or further development of papillary edema. Furthermore, the calf showed evidence of bronchitis, diarrhea and general unthriftiness during this period all of which are evidence of insufficient vitamin A intake. When the carotene intake from alfalfa was increased to 14 micrograms the nyctalopia and bronchitis disappeared and the general appearance of the animal improved.

Calf C399 which received 10 micrograms of carotene per pound of body weight after 425 days of age, plus that in the basal ration making the total intake about 12 to 13 micrograms, maintained plasma carotene at about the 0.2 microgram level. This would indicate that the calf was receiving about the minimum maintenance level. This intake would be just slightly below the minimum requirements set up by Guilbert and Hart (7).

From the results obtained from calves C407, C398, and C404 where the carotene intake was adjusted at 14 micrograms per pound of body weight plus that contained in the basal ration which would make the total intake about 16 micrograms per pound, the level of plasma carotene increased very gradually but never attained very high values. This gradual increase would indicate that some slight storage of vitamin A was taking place at this level. It will be noted that there are individual variations in this respect, C404 remaining below 0.2 microgram per ml. for a considerable period. It was felt throughout the experiment that this calf was always on the verge of vitamin A deficiency.

It will be noted further that at this level of intake while nyctalopia was prevented, the papillary edema in calf C404 showed some increase, C398 was static for a considerable period and C407 showed some decrease. Apparently nyctalopia may be prevented and the outward appearance may be normal at this level of intake but it may not be sufficient to keep the cerebrospinal pressure down to normal levels as indicated by the papillary changes. Data on this particular relationship will be reported at a later date.

In calves C412 and C403 which received 28 micrograms of carotene per pound of body weight the level of plasma carotene rose to as high as 0.7 microgram per ml. of plasma showing considerable contrast to the 14 microgram levels. At this level of intake the calves appeared very thrifty and active. The level of plasma carotene of C400 which received 56 micrograms of carotene per pound of body weight was not any higher than for calves C412 and C403 which received only 28 micrograms and probably indicates individual variation.

By comparing the level of plasma carotene of Calf A25 which received alfalfa hay in liberal quantities with the plasma carotene level of the calves used in this experiment it will be seen that there is considerable difference. In this experiment the plasma carotene values were usually below 0.8 microgram per ml. while Calf A25 attained values up to 3.0 micrograms per ml. during the same period of growth. Mature Holstein cows on pasture may attain carotene values up to 15.0 micrograms per ml. of plasma (8). It would seem therefore that in the bovine the level of blood plasma carotene is subject to wide variations.

The presence of carotene in blood plasma after the removal of the greater part of it from the ration is difficult to explain since the liver is supposed to convert it into vitamin A. It would be reasonable to suppose that shortly after its removal the carotene in the blood plasma would decrease rapidly and be absent in a few days. However this does not appear to be true. In calves C402, C403, and C400 there was a gradual decrease in the level of plasma carotene after the removal of the alfalfa from the ration and not a sudden drop. Unpublished data from mature cows show that when they are removed from a normal ration to a low carotene ration the decrease in blood carotene is gradual. Semb, Baumann and Steenbock (9) suggest that the blood stream can function as a significant storage reserve for carotene.

The results of this investigation show that the carotene requirements reported by Guilbert and Hart (7) for cattle are approximately correct when the criteria of the development of nyctalopia is employed.

It should be kept in mind that the results obtained on the level of plasma carotene were with Holstein and Ayshire calves. Different values on the same level of carotene intake might be expected with the Jersey or Guernsey breed.

SUMMARY AND CONCLUSIONS

1. Calves placed on low carotene rations at 40 to 90 days of age developed nyctalopia in from 48 to 73 days.
2. Papillary edema developed at approximately the same time although there were some variations.
3. An intake of about 9 micrograms of carotene per pound of body weight was not sufficient to prevent nyctalopia or an increase in papillary edema.
4. An intake of about 16 micrograms per pound of body weight was sufficient to maintain the plasma carotene at 0.2 microgram per ml. and above in Holstein and Ayrshire calves.
5. An intake of 16 micrograms per pound of body weight was sufficient to prevent nyctalopia and maintain fair general health.
6. When the plasma carotene values fell below about 0.13 microgram per ml. nyctalopia and papillary edema followed. This value is for Holstein and Ayrshire calves.
7. The carotene requirements of Guilbert and Hart (7) for the bovine are approximately correct where nyctalopia is used as a criterion.

ACKNOWLEDGMENT

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SOME FACTORS AFFECTING THE STABILITY OF CERTAIN MILK PROPERTIES. II. EFFECT OF SUCCULENT ROUGHAGES AND OF STORAGE CONDITIONS ON COLOR*

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Cows' milk contains two classes of yellow pigments one of which is water-soluble and the other fat-soluble. The water-soluble pigment is lactoflavin (riboflavin). It contributes a comparatively small portion to the total of yellow color in milk. The accepted formula (1, 2) indicates that it is an oxidized compound and that it can be easily reduced but not so easily oxidized. It has been shown that comparatively mild oxidizing agents such as bromine, nitric acid and nitrous acid have no effect but that stronger agents such as chromic acid and permanganate do oxidize the compound (3). It would not be expected therefore that very dilute solutions of copper would bring about an oxidation of the molecule.

The fat-soluble pigments of milk belong only to the carotenoid group, and of these, carotene (4) greatly predominates. Probably as much as 10 per cent of the fat pigments (5, 6), however, are xanthophyll (lutein) and perhaps zeaxanthin. Carotenoids are not synthesized by higher animals and, as Palmer and Eckles (4) showed in 1914, the concentration of carotene in milk fat and blood serum varies with the amount of this compound in the feed. However, the amount of carotene (7, 8) which finally is secreted into the milk is relatively low as compared with the amount ingested by the animals. The fact that xanthophyll and zeaxanthin are easily destroyed in the digestive tract (7) of cattle may account for their extremely low concentration in milk.

The eleven double bonds in a conjugated system in the carotene molecule suggest that the molecule might be rather unstable under unfavorable circumstances such as the presence of oxidizing agents. Carotene in ether solution is unstable toward light (9). When freed from its natural state, but still in an impure condition or in solution, it is quite sensitive to heat (5, 10, 11) and quite readily undergoes oxidation in the presence of air. Green plants after being cut and when exposed to sunlight and air show a rapid decrease in carotene content (12). Baur's (13, 14) experiments show that carotenoids take up oxygen in the presence of light by the addition of a large number of moles of oxygen per mole of pigment, resulting in complete bleaching and destruction of the compound. Since carotene is so sensitive to agents which cause oxidation reactions, it would seem that

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subjecting milk to the pasteurizing process would result in a considerable loss of the pigment, especially if some catalyst such as iron or copper were present. Milk, however, loses little, if any, of its vitamin A activity due to pasteurization (15) and since vitamin A and carotene are so closely associated, the implication is that no loss of carotene would occur under the same conditions. The possibility should not be overlooked, however, that the carotene may be split into two molecules of vitamin A by the pasteurizing process. Such a split would result in loss of color but no loss in vitamin potency.

In a previous paper, dealing with the stability of milk flavor, it was shown that a high positive correlation exists between flavor score and yellow color. At the same time the milk flavor studies were being made, data on the stability of color were gathered from the same samples of milk. Two groups of cows, each consisting of two Holsteins and four Guernseys, were fed alternately on beet pulp, corn silage and molasses grass silage as the main roughage ration. The feeding periods were for three weeks but a transition period of one week intervened between the different roughages.

Samples of milk from each cow were taken weekly. Half of each sample was pasteurized before storage while the other half was stored raw. Before storing, however, both the pasteurized and the raw milks were subdivided, half of each getting 1.3 parts of soluble copper per million parts of milk. The raw milk was analyzed on the first, third and fifth days of storage and the pasteurized milk on the second and fifth days for yellow color by the "lactochrometer." The lactochrometer theoretically measures the concentration of all yellow-colored pigments and is not selective for carotene alone.

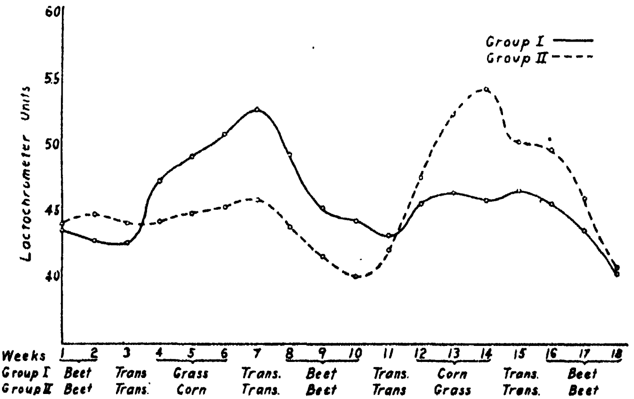

FIG. 1. The week-by-week trend of the yellow color of the milk produced by the two groups of cows.

Figure 1 shows the week-by-week trend of the yellow color of the milk produced by the two groups of cows throughout the experiment. Grass silage and beet pulp have a very pronounced effect on the color, the former producing a milk which by casual inspection of the eye seemed almost as yellow as milk produced on spring pasture; the latter produced a decided decrease in color. The trend of the curves suggests that neither maximum nor minimum effects of the feeds are reached in the comparatively short time of three weeks. This is especially indicated by the fact that milks produced on beet pulp following corn silage reached a lower color level than milks produced on beet pulp following grass silage.

The data presented in Table 1 and summarized in Table 2 show the effect of the three succulent roughages used in this experiment on the yellow color of the milk of each cow. The color values used are those obtained at the end of each feeding period rather than averages for each period. They are probably more representative of each feed since maximum or minimum effects have not been attained during the first and second weeks of each period.

TABLE 1

Effect of roughages on the concentration of yellow color in milk*

Group I	Beet pulp	Grass silage	Beet pulp	Corn silage	Beet pulp
Cow 92	3.90	4.35	4.00	4.00	3.40
" 179	4.50	5.70	4.90	5.10	4.40
" 226	2.55	3.45	2.60	2.90	2.50
" 422	4.85	6.00	5.30	5.50	4.80
" 471	4.85	5.60	4.95	5.20	4.50
" 475	5.05	5.45	4.80	4.85	4.60
Average	4.28	5.09	4.43	4.59	4.03
Group II	**Beet pulp**	**Corn silage**	**Beet pulp**	**Grass silage**	**Beet pulp**
Cow 129	3.95	3.55	3.50	4.30	3.10
" 164	5.20	5.40	4.60	6.20	4.80
" 284	2.25	2.10	1.15	3.20	2.05
" 431	4.55	4.90	4.60	5.60	4.35
" 465	5.70	5.80	5.20	6.80	5.20
" 247	5.25	5.50	5.00	6.50	4.95
Average	4.48	4.54	4.01	5.43	4.08

* Expressed as lactochrometer units.

TABLE 2

Summary of data in table 1

Roughage	Average color
Beet pulp	4.22
Corn silage	4.57
Grass silage	5.26

That milk loses some of its yellow color in storage is indicated by the data in Table 3. The loss, while relatively small, is progressive as shown by the comparative losses on the third and fifth days in storage for raw milk and the second and fifth days for pasteurized milk. Color was not determined immediately after pasteurization but the comparative losses on the fifth day for raw and pasteurized milks indicate that a greater loss occurs in pasteurized milk. Whether or not this greater loss is due to an immediate but small loss during pasteurization or to the inactivation or destruction of some possible color stabilizing factor at that time, thus accelerating subsequent deterioration, remains to be proved.

TABLE 3

Loss of color of milk in storage

Cow no.	Average color* (fresh raw milk)	Loss of color†			
		Raw milk		Pasteurized milk	
		Third day	Fifth day	Second day	Fifth day
465	5.89	2.36	3.36	0.62	2.79
247	5.46	2.46	3.29	2.04	4.04
164	5.36	2.52	4.81	1.50	4.56
422	5.33	2.68	3.27	1.13	3.91
179	5.07	2.39	3.85	1.40	4.12
475	5.06	2.32	2.59	0.53	3.36
471	5.05	3.10	3.17	1.27	3.65
431	4.81	3.49	3.41	2.65	4.60
92	4.11	2.60	2.64	1.75	4.22
129	3.72	4.56	5.69	4.05	6.50
226	2.90	6.89	7.93	4.86	10.66
284	2.23	7.58	8.33	7.03	13.00
Average	4.58	3.58	4.36	2.40	5.45

* Lactochrometer units.
† Expressed as percentage of fresh milk color.

The data in Table 4 show that the presence of soluble copper (1.3 parts per million parts of milk) considerably accelerates the deterioration of color; however, the average loss at the end of the fifth day was less than nine per cent.

TABLE 4

Effect of copper on loss of color

	Percentage loss of color	Difference*
Raw milk		
Third day without copper	3.58	
Third day with copper	5.80	2.22
Fifth day without copper	4.36	
Fifth day with copper	7.06	2.70
Pasteurized milk		
Second day without copper	2.40	
Second day with copper	4.05	1.65
Fifth day without copper	5.45	
Fifth day with copper	8.53	3.08

* Loss without copper subtracted from loss with copper.

Molasses grass silage not only produces a milk higher in yellow color than either beet pulp or corn silage but the data in Table 5 also show that this milk loses less of its total color in storage than that produced by the other two feeds. This is further substantiated by the very high negative coefficients of correlation between original color values and loss of color shown in Table 6. Milks with a high yellow color at the time they are drawn lose a smaller portion of their color in storage than do milks of lower initial color.

TABLE 5

Effect of feed on loss of color

	Loss of color*		
	Beet pulp	Corn silage	Grass silage
Raw milk			
Third day without copper	3.43	4.12	2.48
Third day with copper	5.79	6.04	4.38
Fifth day without copper	4.86	4.01	3.28
Fifth day with copper	7.24	7.09	6.07
Pasteurized milk			
Second day without copper	2.36	2.76	1.93
Second day with copper	4.19	3.68	3.66
Fifth day without copper	5.52	5.36	4.45
Fifth day with copper	8.75	7.75	7.73

* Expressed as percentage of fresh milk color.

The data obtained in this study on the four Holsteins and the 8 Guernseys and presented in Table 7 show that Holstein milk loses more of its color in storage than does Guernsey milk. The data presented are for all samples studied (72 Holstein and 144 Guernsey) and when these data were segregated into milks produced on the three different roughages—beet pulp, corn silage, grass silage—the same relationship held. Addition of copper to these milks emphasized the difference in loss of color.

TABLE 6

Correlation coefficients; color vs. loss of color

	r	S.D.*
Raw milk without copper		
Third day	–.9289 ± .0267†	1.7515 ± .2412†
Fifth day	–.8392 ± .0576	1.8815 ± .2591
Raw milk with copper		
Third day	–.9354 ± .0243	2.9814 ± .4105
Fifth day	–.9241 ± .0284	2.8895 ± .3978
Pasteurized milk without copper		
Second day	–.9268 ± .0275	1.8757 ± .2583
Fifth day	–.9295 ± .0265	2.9737 ± .4094
Pasteurized milk with copper		
Second day	–.9174 ± .0308	2.2312 ± .3072
Fifth day	–.9604 ± .0151	4.5805 ± .6307

Standard deviation of color of fresh samples = 1.0689 ± .1472.

*** S.D. = Standard deviation of loss of color.**

† Probable error.

TABLE 7

Percentage loss of milk color by breeds

	Raw milk		Pasteurized milk	
	Third day	Fifth day	Second day	Fifth day
All samples	3.58	4.36	2.40	5.45
Guernsey samples	2.67	3.47	1.39	3.88
Holstein samples	5.41	6.15	4.42	8.60

The stability of the yellow color in milk appears to be greater than one is led to believe from the known chemical structures of the carotenoids which are present. There is some loss of color in storage and this loss is accelerated to a certain extent by conditions favorable to oxidizing reactions, *i.e.*, pasteurization (heat) and the presence of copper. On the basis of the known comparative stabilities of lactoflavin and carotenoid molecules, it seems reasonable to believe that the loss of color is due to deterioration of one or more of the carotenoids present and not to the lactoflavin. Since Palmer and Eckles (7) have shown that xanthophyll (and possibly zeaxanthin) is less stable in the digestive tract of cattle than carotene, it seems reasonable to expect that they also would be less stable in milk. Therefore, it may be mainly the deterioration of xanthophyll and zeaxanthin that is responsible for the loss of color of milk in storage. Further, since Shrewsbury and Kraybill (16) have postulated the presence of an antioxidant which inhibits or retards oxidation of carotene in butter fat, it may be that xanthophyll and zeaxanthin exhibit an antioxidative action toward carotene. On this basis, oxidation of the carotene in the butter fat would not take place until the antioxidative power of the other two carotenoids had been destroyed. If high-colored milks contain relatively more xanthophyll and zeaxanthin than low-colored milks, then the greater stability of color of high-colored milks may be explained by the above suggested mechanism. This is suggested as a hypothesis, but further work on the point is to be followed.

SUMMARY

The results of this study indicate that:

The feeding of properly ensiled molasses grass silage produces a milk higher in total yellow color than does corn silage or beet pulp; corn silage is somewhat superior to beet pulp in this respect.

There is a small but definite progressive loss of total yellow color of milks in storage. This loss is somewhat accelerated by the pasteurizing process and still more by the presence of soluble copper.

The yellow color of high-colored milks is more stable in stored milks and toward catalytic copper than is that of low-colored milks.

The yellow color of milk produced on molasses grass silage is more stable in storage than is that of milk produced on either beet pulp or corn silage.

Apparently less loss of color occurs in Guernsey than in Holstein stored milk.

An hypothesis is advanced that the loss of yellow color in milk is due to deterioration of xanthophyll and zeaxanthin rather than carotene and that these two carotenoids exhibit antioxidative protection toward carotene in butter fat.

The feeding of molasses grass silage appears to be an excellent means of maintaining a comparatively high level of yellow color in milk during winter months.

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FACTORS AFFECTING ACTIVITY AND HEAT RESISTANCE OF SWISS CHEESE STARTER CULTURES. II. INFLUENCE OF CULTURE MEDIUM*

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During the course of investigations on the heat resistance of a Swiss cheese starter organism, *Lactobacillus helveticus* (2), it became apparent that the inadequacy of the reconstituted skim milk used as the culture medium was largely responsible for frequent loss in activity of the cultures. Similar losses in activity of lactobacillus starter cultures have been encountered repeatedly in the laboratory and in the cheese factory. It is known that certain of the lactic acid-producing bacteria, especially the lactobacilli, are rather fastidious in their nutrient requirements and are sensitive to variations in oxidation-reduction potential. Therefore, a study was made to determine what effect variations in the nutrients and oxidation-reduction potentials of the culture medium might have on activity and heat resistance of *L. helveticus*.

EXPERIMENTAL

Influence of various growth stimulants on rate of acid production by L. helveticus.

The first experiment consisted of a study of the growth of *L. helveticus* in samples of sterile reconstituted skim milk to which had been added various well known growth stimulants. The substances added were: Bactopeptone, neopeptone, yeast water, lactic filtrate,[1] tomato juice and asparagine. The acidity developed within a definite period of incubation was used to indicate the relative growth stimulating ability of the various substances. It was found that all of the substances except asparagine stimulated growth to some extent. However, neopeptone appeared to provide the greatest stimulation, and the Bactopeptone, yeast water, tomato juice and lactic filtrate less stimulation in the order named. Asparagine exerted no significant stimulating effect on growth of *L. helveticus*. From the results it appeared that neopeptone would provide a suitable growth stimulant which could be added to the milk in uniform amounts in order to provide a more favorable medium for growth of *L. helveitcus*. The degree of stimulation produced by neopeptone corresponded roughly to the quantity added and 0.1 per cent was considered sufficient to produce the desired stimulation.

Relationship between age of milk and growth of various organisms.

During the course of investigations to determine the cause of irregular results obtained with *L. helveticus*, it was noted that the age of the milk

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[1] Sterile Berkefeld filtrate of a two weeks old *Streptococcus lactis* milk culture.

apparently influenced the development of this organism. In previous investigations, including those reported in a previous paper (2), the sterile reconstituted skim milk used varied in age from one to eight days and had been kept, for the most part, at room temperature (about 22° to 28° C.).

In order to observe the influence of the age of the milk on the growth of various organisms therein, bottles containing 100 cc. of sterile reconstituted skim milk of varying ages were steamed for ten minutes, cooled to 37° C., and then inoculated with 0.1 per cent of the stock culture of *L. helveticus* organisms. Dilutions were made in an equal volume of two per cent sodium citrate solution. After incubation of the bottle cultures for 10 or 12 hours, titratable acidities and pH values were determined.

Table 1 shows the results obtained by growing various lactic acid producing organisms in samples of milk varying in age from 0 to 22 days. These results clearly indicate the effect of the old and fresh milk on the activity of certain lactobacilli, streptococci and *E. coli.* The older the milk the poorer was the growth of lactobacilli therein. *Str. thermophilus, Str. lactis* and *E. coli* seemed to develop as well in old as in freshly prepared milk.

TABLE 1

Influence of age of milk on rate of acid production by various organisms

Organism	Age of milk at time of inoculation	Hours of incubation at 37° C.	Acid produced
	days		*titratable acidity*
Lactobacillus helveticus	0	12	0.72
	1	12	0.68
	3	12	0.62
	12	12	0.57
Lactobacillus bulgaricus	0	10	0.70
	10	10	0.45
	22	10	0.43
Streptococcus thermophilus	0	12	0.55
	0	12	0.54
	10	12	0.54
	10	12	0.56
Streptococcus lactis	0	12	0.42
	0	12	0.43
	10	12	0.45
	10	12	0.45
			(pH)
Escherichia coli	0	12	5.61
	0	12	5.61
	10	12	5.53
	10	12	5.53

Old milk was steamed for varying lengths of time in order to determine the influence of this procedure on the rate of acid production by *L. helveticus.* It was considered possible that the ten minute period of steaming employed for bottles and flasks of milk might not have been sufficient to drive off all of the dissolved oxygen. However, it was found that when milk was steamed for periods of more than ten minutes, the growth of *L. helveticus* therein was no more active than when a ten minute steaming period was used. The production of acid in milk steamed for 30 minutes was less than in that steamed for ten minutes.

When sterile reconstituted skim milk was stored at 5°–10°, 30° and 45° C., respectively, for one month, growth of *L. helveticus* was most active in that stored at 0° C. and least active in that stored at 45° C.

It might be mentioned that the addition of substances such as neopeptone stimulated growth of *L. helveticus* in both old and in freshly prepared milk.

Oxidation-reduction potentials of old and fresh sterile reconstituted skim milk.

It was considered possible that the old sterile reconstituted skim milk had a higher oxidation-reduction potential than the freshly prepared milk and therefore was less favorable for growth of the lactobacilli. This assumption was supported by the fact that *Str. lactis, Str. thermophilus* and *E. coli* are considered more aerobic than the lactobacilli, and were influenced less by age of milk in which they were grown than were the lactobacilli. Steamed fresh milk was invariably superior to steamed old milk for growth of lactobacilli because steaming old milk did not restore the growth promoting properties to the extent lost in aging. The higher oxidation-reduction potential of the medium which possibly was responsible for poor growth must have been due to factors other than dissolved oxygen.

Oxidation-reduction potentials of milk varying in age from 0 to 22 days were determined. The samples of milk to be tested were placed in 40 cc. quantities in large, eight inch test tubes. They were then steamed for ten minutes and cooled in a water bath to about 25° C. Duplicate platinum electrodes were inserted and the potentials were read with a Leeds and Northrup vacuum tube potentiometer circuit. The electrodes were near the bottom of the tube and were covered with about three and one-half inches of medium. Results obtained by this method may have been relative but they were sufficiently accurate to demonstrate any differences in potentials of the various samples of milk. The results, reported in millivolts, are shown in Table 2.

TABLE 2

Oxidation-reduction potentials of old and fresh milk[1]

Age of milk	Millivolts
days	
0	−70
0	−80
7	+25
7	+40
22	+50
22	+30

[1] All samples of milk were steamed and cooled immediately before use.

They clearly illustrate the difference in oxidation-reduction potentials of fresh and old milk.

Because the oxidation-reduction potential of old milk apparently was higher than that of fresh milk and because, in contrast to the lactobacilli, *Str. lactis*, *Str. thermophilus*, and *E. coli* were rather indifferent to the age of the milk, a comparison was made of the growth of various lactobacilli, and *Str. lactis* and *Str. thermophilus* in fresh milk and in old milk containing varying amounts of neutralized thioglycollic acid. The drop in pH within a given period at a definite temperature was used to compare activity of the organisms in the different samples of milk.

As shown in Table 3, the lactobacilli again grew more rapidly in fresh than in old milk. Old milk containing 0.0001 per cent thioglycollic acid supported growth of lactobacilli almost as well as and in some cases better than did fresh milk. Higher concentrations of thioglycollic acid tended to inhibit growth and lower concentrations apparenty had no great effect on growth. Rate of growth of the streptococci was similar in all of the samples of milk with the exception of those containing .001 per cent thioglycollic acid. *Str. lactis* and *Str. thermophilus* were inhibited far more by a concentration of .001 per cent of the reducing agent than were the lactobacilli. Subsequent determinations of oxidation-reduction potential indicated that when .0001 per cent thioglycollic acid was added to old milk the potential was lowered to a point slightly below that of freshly prepared milk. It is probable that a potential somewhat lower than that of the freshly prepared milk used in these studies would have provided most favorable conditions for growth of the lactobacilli,

TABLE 3

Rate of acid development by certain lactic acid producing bacteria in fresh milk and in old milk containing varying amounts of thioglycollic acid

Organism	Incubation period		Change in pH of milk					
	Time	Temperature	Fresh milk	Old milk plus varying percentages of thioglycollic acid				
				None	.001	.0001	.00001	.000001
	hours	*°C.*						
Lactobacillus helveticus	9	37	1.01	.93	.57	1.16	.92	.83
	11	37	1.62	1.28	.99	1.59	1.28	1.19
	10	37	1.16	1.11	.68	1.58	1.12	1.02
	10	37	1.16	1.11	.44	1.58	1.09	.92
	12	37	1.85	1.38	1.24	1.82	1.50	1.38
Lactobacillus lactis	9	37	.78	.64	.56	.74	.52	...
	12	37	1.57	1.45	1.45	1.72	1.36	1.47
Lactobacillus	9	37	1.41	1.10	.71	1.20	1.15	1.07
	12	37	2.29	2.03	1.24	2.16	2.08	....
Streptococcus lactis	9	28	.72	.91	.00	.81	.93	.86
Streptococcus thermophilus	9	37	1.53	1.54	.03	1.54	1.53	1.53
	12	37	1.70	1.74	.33	1.84	1.87	1.80

for further studies have demonstrated that addition of thioglycollic acid and sodium thiosulfate stimulated growth of various lactobacilli to some extent in freshly prepared reconstituted skim milk. Though they do not eliminate the possible nutritional effect of the reducing substances added to the milk, in general, the results indicate that the oxidation-reduction potential may be one of the factors responsible for the difference in the ability of samples of old and fresh milk to support growth of lactobacilli, and this difference in potential is not due to a difference in the amounts of dissolved oxygen from the air.

Different lots of milk powder may vary considerably and later studies have shown that the lot of skim milk powder used to prepare the reconstituted milk may significantly influence the effect of age of milk on growth of lactobacilli.

Influence of culture medium and time and temperature of incubation on the ability of L. helveticus to develop at 44° C. following heating at 60° C. for thirty minutes.

The discovery that milk only one week old was decidedly inferior to fresh milk for growth of lactobacilli explained many of the irregularities previously encountered when no attention was paid to age of the milk used.

In order to determine the combined influence of culture medium and time and temperature of incubation on activity and heat resistance of *L. helveticus* cultures of this organism were carried in a poor medium (milk one week old) and in a milk which favorably supported growth. According to results reported above, such a favorable medium could be provided by the addition of 0.1 per cent of neopeptone to the reconstituted milk before sterilization. This medium was always prepared not more than one or two days before use.

The methods used to carry mother cultures, prepare inoculating cultures and compare activity of cultures after heat treatment were similar to those outlined in a previous paper (2). Duplicate mother cultures were grown for periods of 12, 14, and 16 hours, respectively, every day at both 37° and 40° C. in each type of medium. The cultures were inoculated daily at 4:00, 6:00 and 8:00 P.M., so that their respective incubation periods ended at 8:00 A.M. the following morning. At this time they were placed at 10° C. until time for the next daily transfer. A preliminary experiment had demonstrated that cultures could be stored at 10° C. for at least 12 hours each day without significantly influencing their activity or heat resistance. The method used in carrying the mother cultures was intended to provide cultures at 37° which were just as mature as certain of the 40° C. cultures, so that the factor of maturity would not complicate any comparisons of the direct effect of incubation temperature on ability to develop following heating. In order to maintain conditions as nearly uniform as possible, whenever a culture was to be transferred, the inoculum was first diluted with an equal volume of two per cent sodium citrate solution. All tubes were at a temperature of 37° C. when inoculated.

For a period of two weeks inoculating cultures were prepared about every second day and these then transferred to flasks, heat shocked at 60° C. for 30 minutes, and then incubated at 44° C. All of the milk contained in the flasks consisted of sterile, freshly prepared reconstituted skim milk plus 0.1 per cent neopeptone. The rate of acid production by the various cultures was followed by pH determinations at hourly intervals for the first ten hours and at the 24th hour after heating. At the end of the fifth transfer in the poor medium, the cultures were so weak and run down that in certain cases a temperature of 37° rather than 44° C. was employed for incubation of cultures following heating. This reduction in incubation temperature allowed the organisms to develop sufficiently to demonstrate any differences in activity between cultures.

Table 4 shows the results obtained in this experiment. Instead of including all of the pH readings made during incubation following heat treatment, only the drop in pH during the first seven hours following heating is shown. The quantity of acid produced by a culture during the first seven hours after heat treatment indicates the ability of that culture to withstand exposure to heat and to develop after heating.

The most obvious effect of growth in the poor medium was the gradual decrease in activity of the cultures, irrespective of the time or temperature of incubation. As transfers in the old medium were continued, the titratable acidities of the inoculating cultures became lower. At the same time the activity of the cultures following heating also steadily decreased as transfers in old milk were continued, until finally their development at 44° C. was so negligible that it was necessary to lower the temperature of incubation following heating. When the temperature of incubation following heating was lowered, another gradual change was observed. At the time of the first transfer the 40° cultures were less heat resistant than the 37°. However, by the twelfth transfer the 40° cultures were apparently the more resistant. The 16 hour 37° and 12 and 14 hour 40° inoculating cultures had about the same titratable acidity, yet the 40° was the more resistant. The differences in activity following heating were not great, but they were fairly consistent.

The increase in titratable acidities (table 4) of the respective inoculating cultures in the favorable medium is indicative of the increased activity of these cultures after successive transfers in this medium. At the same time, certain changes in the heat resistance of the cultures were being induced at the incubation temperatures used. After one transfer, the 16 hour, 37° culture was the most heat resistant, then came the 14 hour, 37° and 12 hour, 40° cultures. Least resistant were the 12 hour, 37° and 16 hour, 40° cultures. The titrations indicated that the 12 hour, 37° culture was not mature enough and the 16 hour, 40° culture was too old to demonstrate as much activity as that shown by the other cultures.

TABLE 4

Influence of culture medium and of time and temperature of incubation on the ability of Lactobacillus helveticus to develop at 44° C. following heat shocking at 60° C. for thirty minutes

Incubation period		Culture medium	Number of transfer of mother culture used to prepare inoculating culture											
Time	Temperature		1	3	5	8	10	12	1	3	5	8	10	12
hours	°*C.*		*titratable acid of inoculating culture #*						*drop in pH following heat shocking #*					
12	37	Old milk	0.93	0.90		0.85	0.73	0.80	.24	.16		.08	.02	.11*
14	37		1.03	1.00	0.96	0.92		0.91	.25	.15	.08	.08		.21*
16	37		1.10	1.08	0.99	1.01	0.95	0.96	.28	.12	.08	.04		.17*
12	40		1.05	1.05		1.00		0.97	.18	.16		.08		.25*
14	40		1.15	1.17	1.10	1.10		1.02	.20	.11	.09	.10		.28*
16	40		1.23	1.24	1.15	1.14	1.08	1.06	.20	.17	.08	.08		.21*
12	37	Fresh milk[1]	0.91	0.99	1.00	1.04	1.04	1.04	.46	.46	.38	.24	.51	.54
14	37		1.02	1.09	1.10	1.10	1.12	1.12	.58	.61	.44	.28	.48	.51
16	37		1.10	1.17	1.20	1.15	1.20	1.21	.64	.50	.43	.27	.46	.42
12	40		1.03	1.11	1.09	1.08	1.10	1.14	.56	.42	.38	.27	.37	.35
14	40		1.13	1.19	1.19	1.17	1.18	1.20	.48	.44	.37	.28	.32	.33
16	40		1.19	1.24	1.24	1.28	1.24	1.27	.50	.40	.43	.31	.38	.36

Table shows titratable acidities of respective inoculating cultures and drop in pH during the first seven hours following heat shocking of the respective inoculating cultures.

* Because they were so weak, these cultures were incubated at 37° C. rather than 44° C. following heat shocking.

[1] Fresh milk contained 0.1 per cent of neopeptone added before sterilization.

By the end of the tenth transfer in the favorable medium, all of the 37° were more heat resistant than any of the 40° cultures. The 14 and 16 hour, 37° were more active than the 12 and 14 hour, 40° cultures, respectively, although titratable acidities indicated that the inoculating cultures had attained approximately equivalent maturities. The most active following heating was the 12 hour, 37° culture.

The results of a subsequent experiment demonstrated that the activity of any one culture at 44° C. following heat treatment was almost directly proportional to the size of inoculum and therefore proportional to the number of active cells present. It is probable that in the experiments reported in the present studies, the ability to develop at 44° C. after heating depended more on the number of active cells of *L. helveticus* present than on any adaptability to growth at a high temperature such as 44° C. The number of active cells was undoubtedly greater in cultures grown in freshly prepared milk than in those grown in old milk.

DISCUSSION

According to the results reported above and those of other investigators, the addition of substances such as peptone, tomato juice, lactic filtrate, yeast water and liver extract stimulates growth of lactobacilli in milk. In the present studies no attempt was made to determine the actual purpose which such substances serve.

The observation that aging of sterile reconstituted skim milk lowers its ability to support active growth of lactobacilli was a significant one, because frequently mother starters or bulk cultures have been grown in milk which had been sterilized and held for varying periods at room temperature. The limited studies reported above indicate that the higher oxidation-reduction potential of older milk was responsible, at least in part, for the lessened growth of the lactobacilli. Since the more aerobic organisms such as *Str. lactis, Str. thermophilus* and *E. coli* grew as well in old as in freshly prepared milk, while the lactobacilli grew better in the fresh milk, it was concluded that the higher oxidation-reduction potential of older milk was one of the factors limiting development of lactobacilli. Therefore it was not surprising that lowering the oxidation-reduction potential by addition of neutralized thioglycollic acid aided the growth of lactobacilli but not that of the streptococci. Observations not reported in this paper, obtained in use of skim milk-tryptone agar indicated, also, that as the agar aged, it gradually became a poorer culture medium for lactobacilli. Additional studies are necessary to more definitely establish the influence of the oxidation-reduction potential of the agar culture medium on initiation of growth by the lactobacilli.

Considering the results as a whole it becomes apparent that certain measures may be taken to maintain or increase the activity of stock or starter cultures of lactobacilli such as those used in the present studies. First, certain growth stimulants may be added to the milk in order to improve its growth promoting properties. Second, freshly prepared milk obviously provides a more favorable medium than old milk. Third, in certain types of milk, adjusting the oxidation-reduction potential to a level more favorable for growth may improve its ability to support growth. The use of such methods should insure active cultures in a milk medium similar to that used in these studies.

The medium in which *L. helveticus* was grown definitely played a part in the effect of incubation temperature on activity following heat treatment, and apparently the influence of the medium overshadowed the influence of incubation temperature on activity following heat shocking of starter organisms such as *L. helveticus*. When cultures of *L. helveticus* were grown for 12, 14 and 16 hours at 37° and 40° C., respectively, in a poor and in a favorable medium, cultures with about equivalent maturities were obtained at the two temperatures. Results with these cultures demonstrated that in a poor medium growth at the higher temperature resulted in greater heat resistance. On the other hand in a favorable medium, where growth was more rapid, incubation at 37° resulted in greater heat resistance than incubation at 40° C.

It has been suggested by Elliker and Frazier (1) that rapid growth of *E. coli* in a favorable medium may result in a greater accumulation of mature, heat resistant cells during the maximum stationary phase of growth than may result from slow growth in a poor medium. Similar factors may be responsible for effect of incubation temperature on heat resistance of *L. helveticus.*

Very striking was the gradual decrease in activity on continued transfer in the poor medium in contrast to the gradual increase in activity and heat resistance in the favorable medium. These results suggest that in many cases the gradual decrease in activity of certain lactobacillus starter cultures may be due to continued growth in an unfavorable medium.

Also demonstrated was the fact that a difference in incubation time of two or four hours around the period of maximum heat resistance of *L. helveticus* definitely influenced its heat resistance. Successive daily incubation of the cultures for their respective incubation periods as compared to one incubation period emphasized the effect of incubation time.

SUMMARY

1. *L. helveticus, L. lactis* and *Lactobacillus* sp. all developed more actively in freshly prepared than in old, sterile reconstituted skim milk. The age of the sterile reconstituted skim milk used as culture medium had no significant influence on growth of *Str. thermophilus, Str. lactis* and *E. coli.*

2. Substances such as peptone, tomato juice, yeast water and lactic fil-

trate stimulated growth of *L. helveticus* in both old and freshly prepared sterile reconstituted skim milk.

3. The oxidation-reduction potential of steamed, freshly prepared, sterile reconstituted skim milk was significantly lower than that of steamed, old sterile reconstituted skim milk. Addition of a reducing substance, neutralized thioglycollic acid, to old sterile reconstituted skim milk stimulated development of certain lactobacilli but did not significantly stimulate development of *Str. lactis*, *Str. thermophilus* and *E. coli*.

4. A succession of transfers of *L. helveticus* in old sterile reconstituted skim milk resulted in a gradual decrease, while a succession of transfers in a milk more favorable for growth resulted in an increase in their activity and in their ability to develop at 44° C. following heating. When cultures of *L. helveticus* were carried at 37° and 40° C., respectively, for numerous successive transfers in a favorable medium, those grown at 37° were more active following heat treatment than were those grown at 40° C. However, when cultures were carried in the same manner in a poor medium, those grown at 40° were more active following heating than were the 37° C. cultures.

5. When stock or starter cultures of lactobacilli similar to those used in these investigations are grown in milk of the type used in these studies, their activity may be insured if they are carried in freshly prepared milk, in milk in which the oxidation-reduction potential has been adjusted to a level favorable for initiation of growth, or in milk to which growth stimulants have been added.

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THE RELATION OF THE REFRACTIVE INDEX OF EVAPORATED AND CONDENSED MILK SERUM TO THE TOTAL SOLIDS CONTENT

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The refractive index of milk serum has been used primarily for the detection of added water in milk. Since the results of this determination are influenced directly by the soluble components it is logical to expect a definite relationship to exist between the total solids and the refractive index of the serum.

Some investigators have shown that a definite correlation does exist and a few have attempted to make a practical application of this relationship.

Wiegner (1) found that the specific gravity of the dry constituents of the calcium chloride serum is nearly constant at 1.6850. He shows that a definite relation exists between total solids, specific refractive index and density.

Rice and Miscall (2) used an Abbé refractometer to establish a correlation between the refractive index and total solids of several concentrated milk products. They report satisfactory results except with products containing a high fat content and those in which lactose crystallized out due to concentration.

These authors determined the total solids and refractive index for each sample of product they examined. The method of least squares was applied to these data, and formulas were developed and used for computing the total solids in evaporated and sweetened condensed skim milk. According to Rice and Miscall the total solids of these products may be computed very accurately by using the formulas developed for them.

The purpose of the present investigation was to study the relationship between the total solids and the refractive index of the milk serum from the products used in these experiments and to develop, if possible, a rapid routine determination for total solids.

DESCRIPTION OF APPARATUS

Samples of condensed milk products were secured from the University Creamery and others were condensed in the laboratory.

The laboratory condensing equipment consisted of a 12-liter flask immersed in a hot water bath and connected to a liter suction flask by a four-

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foot condenser. The suction flask was connected directly to a water vacuum pump and arranged so that vapors and condensate passed out through the connecting line and water pump into the drain.

A Zeiss Dipping Refractometer equipped with prism No. 1 was used to determine refractive indices. This instrument was standardized with pure water at 20° and all readings were made at this temperature.

The copper sulphate solution for preparing the serum was made according to the Official Method (3) ("72.5 grams $CuSO_4 \cdot 5H_2O$ per liter, adjusted if necessary to read 36 at 20° on the scale of the Zeiss immersion refractometer, or to a specific gravity of 1.0443 at 20/4°").

The total solids were determined by the Official Method (3). The samples were weighed into lead foil dishes 5.5 cm. in diameter, each containing approximately 10 grams of ignited sand.

EXPERIMENTAL

The samples of the concentrated milk products to be examined were adjusted to temperature of 20° as soon as they were removed from the evaporating unit. The total solids and the refractive index of the serum were determined immediately.

To prepare the serum, fifty grams of the product to be examined were weighed on a sensitive balance. In some experiments the samples were diluted with water and copper sulphate solution was added; in others copper sulphate solution alone was added to precipitate the proteins and to provide enough serum for a refractometer reading. The latter procedure is preferred because the errors introduced by dilution are minimized. The amount of coagulant added and any dilution with water is indicated at the top of each table.

The acetic serum method was used to a limited extent but it was discontinued because the milk proteins were not completely precipitated. This method is also objectionable because it requires too much time. Other methods for preparing the serum were tried but were not used because they appeared to have no evident advantages over the copper sulphate method.

The formulas used for computing the total solids in condensed skim milk were developed as follows:

Samples of skim milk were taken from the evaporating pan at various intervals in order to have a series of samples of varying concentration. The scale reading and total solids were determined for each sample including the original skim milk. The method of least squares was applied to a number of these determinations and the regression equation developed. This was used as a formula for computing the total solids in the samples examined. By using this formula the total solids in any sample of concentrated skim milk may be computed if the dipping refractometer scale reading at 20° is known for the serum from the sample.

Using the same procedure a formula was developed for computing the total solids in evaporated milk.

The scale reading is used in the calculations because it is obviously more convenient to use than the refractive index.

ANALYTICAL RESULTS

Tables 1 to 4 summarize the results of four experiments. The corresponding plates show the plotted points (total solids—scale reading) with their regression lines.

The formula derived from each experiment is shown at the end of each table following the summary of the statistical calculations.

The determined total solids values are the averages of duplicate determinations.

TABLE 1

Condensed skim milk; 50 gm. of sample, 50 gm. of water and 25 ml. of copper sulphate

Scale	nD	Determined T.S.	Computed T.S.	Error
28.25	1.33829	9.20	9.76	0.56
29.00	1.33857	10.95	10.46	- 0.49
32.40	1.33986	14.46	13.66	- 0.80
52.45	1.34741	33.76	32.50	- 1.26
29.55	1.33878	10.64	10.98	0.34
33.00	1.34009	13.86	14.22	0.36
38.20	1.34206	18.77	19.11	0.34
42.00	1.34350	22.15	22.68	0.53
44.70	1.34452	24.75	25.22	0.47
49.60	1.34635	29.06	29.82	0.76
28.30	1.33830	9.24	9.81	0.57
28.90	1.33853	10.58	10.37	- 0.21
30.70	1.33922	12.51	12.06	- 0.45
28.10	1.33823	9.34	9.62	0.28
29.00	1.33857	10.95	10.46	- 0.49
30.60	1.33918	12.50	11.97	- 0.53

Summary of calculations of table 1

Correlation coefficient	0.9970
Mean of scale readings	34.6719
Mean of total solids	15.7950
Std. deviation of scale readings	7.8853
Std. deviation of total solids	7.4337
Std. error of estimate	0.4641

Formula—T.S = 0.9398 (Scale reading at 20°) - 16.7913.

DISCUSSION

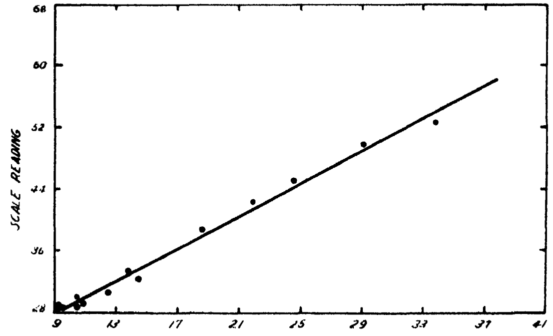
An examination of Tables 1 to 4 and the corresponding plates offers sufficient evidence to prove that the relationship between the refractive index and the total solids is linear.

A preliminary experiment in which condensed skim milk was used showed that this relationship existed but the data secured were somewhat irregular. Later experiments indicate that some of the errors introduced were due to the total solids determinations. In the preliminary experiment

the solids were determined without the use of sand and the samples were considerably caramelized before they came to constant weight. The errors in the determinations were indicated by the unsatisfactory checks secured with duplicate determinations. This difficulty was eliminated by adding sand to the solids dishes to facilitate evaporation. Excellent checks were secured by this procedure.

Another source of error is the variation in the total solids and the refractive index of the serum from the original or uncondensed milk examined

PLATE-I



GRAPH OF DATA IN TABLE 1 WITH REGRESSION LINE

TABLE 2

Condensed skim milk; 50 gm. of sample and 50 ml. of copper sulphate

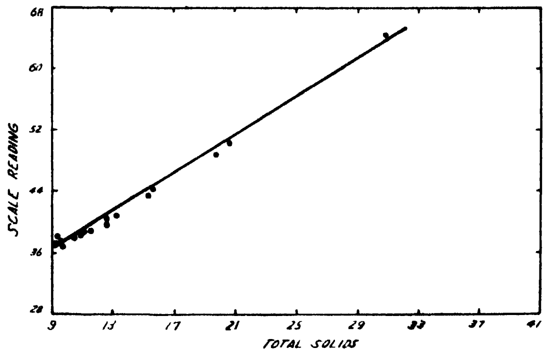
Scale	nD	Determined T.S.	Computed T.S.	Error
37.60	1.34183	9.24	10.02	0.78
38.30	1.34209	10.58	10.58	0.00
40.70	1.34301	12.51	12.50	−0.01
37.00	1.34161	9.34	9.54	0.20
38.50	1.34217	10.95	10.74	−0.21
40.45	1.34291	12.50	12.30	−0.20
44.28	1.34436	15.47	15.36	−0.11
50.58	1.34672	20.49	20.39	−0.10
37.50	1.34180	9.52	9.94	0.42
38.90	1.34232	11.36	11.06	−0.30
40.80	1.34304	13.06	12.57	−0.49
43.60	1.34410	15.38	14.82	−0.56
49.00	1.34613	19.71	19.13	−0.58
64.40	1.35183	30.88	31.44	0.56
37.55	1.34181	9.35	10.00	0.65
38.80	1.34228	11.04	10.98	0.06

Summary of calculations of table 2

Correlation coefficient	**0.9985**
Mean of scale readings	**42.3725**
Mean of total solids	**13.8363**
Std. deviation of scale readings	**6.9174**
Std. deviation of total solids	**5.5357**
Std. error of estimate	**0.3022**

Formula—T.S. = 0.79907 (Scale reading at 20°) − 20.0224.

PLATE 2



GRAPH OF DATA IN TABLE 2 WITH REGRESSION LINE

TABLE 3

Evaporated Milk; 50 gm. sample and 25 ml. copper sulphate

Scale	nD	Determined T.S.	Computed T.S.	Error
37.80	1.34191	13.01	13.77	0.76
40.25	1.34284	16.13	15.64	– 0.49
44.70	1.34452	19.53	19.08	– 0.45
51.95	1.34722	24.82	24.59	– 0.23
60.00	1.35021	30.18	30.75	0.57
37.80	1.34191	12.85	13.77	0.92
38.72	1.34225	14.83	14.47	– 0.36
41.00	1.34312	16.75	16.22	– 0.53
44.00	1.34425	19.00	18.51	– 0.49
48.60	1.34598	22.51	22.03	– 0.48
55.30	1.34847	27.06	27.15	0.09
37.90	1.34194	12.88	13.85	0.97
38.50	1.34217	14.44	14.30	– 0.14
41.00	1.34312	16.52	16.22	– 0.32
44.00	1.34425	18.83	18.51	– 0.32
48.00	1.34575	21.69	21.57	– 0.12
54.50	1.34818	26.31	26.54	0.23
37.50	1.34180	12.83	13.54	0.71
37.82	1.34191	14.11	13.78	– 0.33
40.10	1.34277	15.88	15.53	– 0.35
42.90	1.34383	18.06	17.67	– 0.39
47.40	1.34553	21.45	21.11	– 0.34
52.00	1.34724	24.65	24.63	– 0.02
58.35	1.34959	28.96	29.49	0.53
37.60	1.34183	13.02	13.62	0.60

Summary of calculations of Table 3

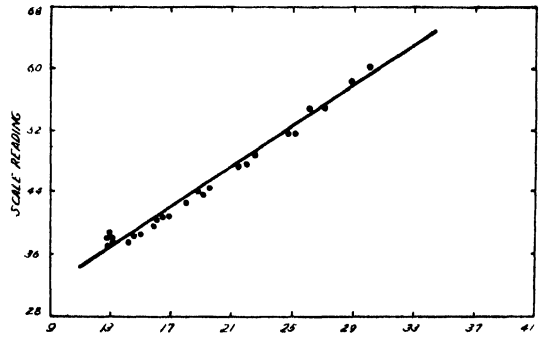
Correlation coefficient	**0.9958**
Mean of scale reading	**44.7076**
Mean of total solids	**19.0520**
Std. deviation of scale reading	**6.9451**
Std. deviation of total solids	**5.3342**
Std. error of estimate	**0.49**

Formula—T.S. = 0.7648 (Scale reading at 20°) – 15.1405.

(skim milk and whole milk containing 4 per cent fat). The formulas derived for computing the total solids would be more accurate if these variations were excluded from the determinations used when computing regression equations. Undoubtedly the most satisfactory samples for an investigation of this nature would be those containing from 10 to 25 per cent total solids.

In each table the formula for computing the total solids was developed from the data presented and was used to compute the total solids in that

PLATE 3



GRAPH OF DATA IN TABLE 3 WITH REGRESSION LINE

TABLE 4

Canned evaporated whole milk (purchased on market) 50 gm. sample and 25 ml. copper sulphate

Scale	nD	Determined T.S.	Computed T.S.	Error
53.70	1.34788	25.71	25.87	0.16
56.60	1.34895	25.73	26.23	0.50
54.95	1.34834	26.13	26.03	−0.10
54.50	1.34818	26.27	25.97	−0.30
56.00	1.34873	26.14	26.16	0.02
55.10	1.34840	26.42	26.05	−0.37
56.15	1.34879	26.24	26.17	−0.07
56.90	1.34896	25.96	26.27	0.31
55.00	1.34836	26.00	26.04	0.03
55.00	1.34836	26.30	26.04	−0.27
55.75	1.34864	26.71	26.12	−0.58
55.75	1.34864	26.17	26.12	−0.04
55.80	1.34866	26.18	26.13	−0.05
57.10	1.34914	25.72	26.29	0.57
53.71	1.34788	25.88	25.88	0.00
54.35	1.34812	25.75	25.95	0.20

Summary of calculations of table 4

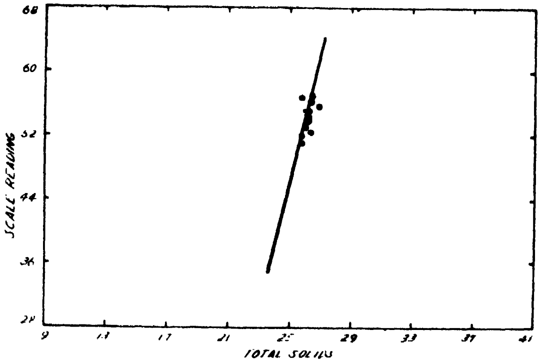
Correlation coefficient	0.4486
Mean of scale reading	55.3975
Mean of total solids	26.0819
Std. deviation of scale reading	1.0056
Std. deviation of total solids	0.2745
Std. error of estimate	0.2453

Formula—T.S. = 0.122446 (Scale reading at 20°) + 19.2986.

table. This does not demonstrate the accuracy of the formula for computing the total solids of all samples of condensed skim or exaporated milk.

In order to show how accurately the total solids may be computed for any sample of condensed skim milk, the formula derived from the data in Table 2 is used to compute the total solids in a number of samples of condensed skim milk examined in the preliminary experiments.

PLATE 4



GRAPH OF DATA IN TABLE 4 WITH REGRESSION LINE

TABLE 5

Total solids in the preliminary experiment computed by the formula derived from data in table 2

Scale	Determined T.S.	Computed T.S.	Error
28.69	9.26	10.17	0.91
36.46	16.86	17.47	0.61
40.00	20.22	20.80	0.58
50.50	28.36	30.67	2.31
55.71	33.40	35.57	2.17
62.70	38.19	42.13	3.94
28.25	9.12	9.76	0.64
31.00	12.51	12.34	-0.17
38.90	20.49	19.77	-0.72
56.95	36.16	36.73	0.57
28.45	9.29	9.95	0.66
33.00	13.96	14.22	0.26
38.51	19.31	19.40	0.09
43.10	23.64	23.71	0.07
47.00	26.46	27.38	0.92
60.20	37.37	39.78	2.41
28.20	9.35	9.71	0.36
28.25	10.26	9.76	-0.50
32.40	14.58	13.66	-0.92
41.70	24.30	22.40	-1.90
51.95	33.72	32.03	-1.69
28.25	9.20	9.76	0.56
29.00	10.95	10.46	-0.49
32.40	14.46	13.66	-0.80
52.45	33.76	32.50	-1.26

To the samples in Table 2, fifty ml. of copper sulphate were added in order to precipitate the proteins completely and to eliminate the preliminary addition of water. The satisfactory results obtained in this experiment are attributed to the direct addition of copper sulphate to the product.

The application of the formula derived from the data in this table is demonstrated by using it to compute the total solids in samples of concentrated skim milk. The samples were prepared by using plain condensed skim and diluting portions of this with skim milk in order to have a series of samples of different total solids content. The total solids and the refractive index of the serum were determined for each of these samples and the total solids were computed by the formula. The results are as follows:

Scale	nD	Determined T.S.	Computed T.S.	Error
37.70	1.34187	9.38	10.10	0.72
61.61	1.35081	26.45	29.21	2.76
56.76	1.34901	23.25	25.33	2.08
68.65	1.35338	31.22	34.83	3.61
60.45	1.35038	25.92	28.28	2.36
44.10	1.34429	14.35	15.21	0.86
54.60	1.34821	21.71	23.61	1.90

These results need little explanation. The errors in the computed total solids are rather large if they are considered from a quantitative point of view.

The data in Table 4 were obtained by the analysis of canned evaporated milk secured at various local markets. It is evident that the formula derived from the data in this table cannot be used to compute the total solids of any

TABLE 6

Total solids in table 4 computed by the formula derived from the data in table 3

Scale	Determined T.S.	Computed T.S.	Error
53.70	25.71	25.93	0.22
56.60	25.73	28.15	2.42
54.95	26.13	26.89	0.76
54.50	26.27	26.54	0.27
56.00	26.14	27.69	1.55
55.10	26.42	27.00	0.58
56.15	26.24	27.80	1.56
56.90	25.96	28.38	2.42
55.00	26.00	26.92	0.92
55.00	26.30	26.92	0.62
55.75	26.71	27.50	0.79
55.75	26.17	27.50	1.33
55.80	26.18	27.54	1.36
57.10	25.72	28.53	2.81
53.71	25.88	25.94	0.06
54.35	25.75	26.43	0.68

other sample of evaporated milk unless it has approximately 25 per cent total solids. It is used to compute the total solids of the samples in this table.

In order to demonstrate the application of a formula for evaporated milk, the formula derived from the data in Table 3 is used to compute the total solids in Table 4.

Several discrepancies are evident in the data. The total solids content remains rather constant while there is considerable variation in the refractometer readings.

For comparison the formula derived from the data in Table 4 is used to compute the total solids of the samples in Table 3 that have approximately 25 per cent total solids.

Scale	Determined T.S.	Computed T.S.	Error
51.95	24.82	25.67	0.85
60.00	30.18	26.64	-3.54
55.30	27.06	26.07	-0.99
54.50	26.31	25.97	-0.34
52.00	24.65	25.67	1.02
58.35	28.96	26.44	-2.52

Of the experiments presented, the evaporated milk is the most interesting. The uniform composition of the commercial product is well illustrated by Table 4 and Plate Number 4. The regression equation for this product as well as for others may have an added significance if derived from a greater number of determinations. A minimum number of samples have been examined in these experiments and this may be the cause of some of the irregularities noted.

The variation in the composition of normal milk and factors such as heat, standardization and acidity that bring about changes in the chemical composition must also be considered. The development of acidity in a product is one of the most important problems. Useful refractometer readings can be made only on fresh products. Further investigation may show that some of the above factors will limit the application of the theoretical principles involved in this discussion. A suitable formula may be developed for computing the total solids in some products by deriving the regression equation from a greater number of determinations and possibly using a correction factor.

Further investigation may prove that milks whether from an individual cow or a herd, must be considered separate colloidal systems. Any physical constant determined on an individual sample is an expression of the complex peculiarities of that sample and it does not imply that this constant can be applied to any sample chosen at random.

CONCLUSIONS

1. Sufficient evidence is presented to show that a linear relationship exists between the total solids and the refractive index of the serum of evaporated and condensed skim milk.

2. The formula developed for one type of product cannot be used to compute quantitatively the total solids in every sample of that product.

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THE DETERMINATION OF CHLORIDE IN MILK

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The determination of chloride in milk is one of the standard tests used in the diagnosis of mastitis in dairy cows. The methods most commonly used in this country are modifications of Mohr's direct titration method, in which chloride is titrated with a standardized solution of silver nitrate in the presence of potassium chromate indicator. Direct titratable chloride values above 0.14 (20) or 0.16 (11) per cent have been cited as signifying abnormality in milk caused by a diseased condition of the udder. In an intensive study of the mastitis problem in these laboratories, covering a period of several years and involving tests on more than 3000 quarter-udder samples taken periodically throughout several lactations, our results have indicated that in the vast majority of samples, when a strictly quantitative method for chloride is used, 0.12 per cent chloride is a surer criterion of mastitis than the values quoted above. We shall publish later a paper containing evidence that the chloride test, when carried out quantitatively on quarter-udder samples, gives a very useful indication of mastitic conditions in the udder. In much of the diagnostic work done in the past, investigators apparently have been at a disadvantage because of their use of methods that are not sufficiently precise. Because of lack of precision apparent in the direct titration method, the writer found it desirable at an early stage in the mastitis work to survey the literature and conduct experiments in an attempt to find a method which would not be too laborious and which would be sufficiently precise to detect small changes in chloride content, as well as small differences in chloride values between different quarters.

REVIEW OF LITERATURE

The direct titration of chloride with silver nitrate and with potassium chromate indicator, in neutral chemical solutions in which interfering substances are absent, was proposed by Mohr (14) in 1861. His data indicate that a slight excess of silver nitrate is always required to produce a visible endpoint. His method has been applied to the chloride test in milk by Richmond (18), Hammer and Bailey (8), Rosell (20), Sharp and his coworkers (24), and others. In Hammer and Bailey's method, 10 volumes of water are added to 1 volume of milk before the titration, and the titration is carried out in a white porcelain dish in order to make the endpoint more visible. They reported that, although the direct titration gave results which were always higher than those obtained by titrating chloride in ashed samples, it was "a satisfactory index of the chloride content."

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Hammer and Bailey found that the presence of casein is an important cause of positive error in the direct titration, an error which Poetschke (16) attributed largely to the presence of phosphates and to the evident fact that the endpoint is very indefinite. Poetschke reported much higher results by direct titration than by the ashing method; while he found that the positive error was reduced considerably and the visibility of the endpoints increased by titrating undiluted rather than diluted samples, the endpoints were still so indistinct that it was impossible to secure satisfactory results.

Sharp and his co-workers (24) devised a direct titration method in which samples were titrated without first being diluted with water. They found that the positive error was relatively great when the amount of indicator used was small and when water was added before titration. They found that the endpoints were not satisfactory and they stated that, since the extent of the positive error was not known, values obtained by direct titration should be referred to as "direct titratable chloride values" rather than as percentages of chloride. Blood and Rowlands (2) stated that the percentage found by direct titration was always absurdly high; high results have been likewise reported by everyone who has made comparisons, including Weiss (33), Meyer (13), Drost (7), and others. W. L. Davies (6), who used and recommended a wet digestion and Volhard titration method, stated that the high results obtained by direct silver nitrate titration in milk are caused by chemical combination of silver ions with protein.

Recently the use of an adsorption indicator, dichlorofluorescein, has been proposed by Kolthoff *et al.* (12) for direct titrations with silver nitrate. The color of a clear solution changes from a fluorescent greenish hue to a bright pink when the endpoint is reached, and the result in a clear solution appears to be strictly quantitative when interfering substances are not present; however, Collier (4) has shown that, in biological materials, the pH of the sample has an influence on the endpoint, and should be 7.0 to 7.5. Rose (19) studied the use of this indicator for titrating chloride in milk, and reported that accuracy of results not only depends upon pH but is influenced by the presence of protein. If more than 3 per cent protein was present there was a proportional positive error and it was necessary to remove the protein before titrating. Hastings and Beach (10) state that the ashing method gives the lowest and most accurate values, the use of dichlorofluorescein gives somewhat higher results, and the use of chromate gives the highest values. They believed that a wet ashing method would be best for quantitative work.

Of the strictly quantitative methods, the Volhard (30) titration has been used generally in physiological studies and has been used in analyses of milk principally by foreign workers. It has been demonstrated that, since milk does not contain significant amounts of colored substances which might interfere, it is feasible to omit the wet digestion procedure and thus materially shorten the procedure for determining chloride accurately by the Volhard method. Moreover, it has been shown that the reagents added before titrating may be combined, thus further simplifying the method.

In such a procedure, as proposed for urine studies by Strauss (26), a standardized solution of silver nitrate containing nitric acid and ferric ammonium sulfate (ferric alum) indicator, known as the "Martius-Lüttke solution," is added to the test material and the mixture is titrated with standardized potassium sulfocyanate. Drost (7) has shown that the presence of proteins and other constituents in milk does not affect the results. He, and also Munchberg (15), obtained the same results in undigested milk samples by using this method as by using other quantitative methods. Although the writer has found no reference in the literature of this country to the Volhard titration of undigested milk, it is described in foreign literature as the simplest and quickest of the accurate methods (22) (23) (13). A modification of such a method has been published recently, in abstract form, by the writer (21).

In a consideration of the chemical factors on which the usefulness of this method in milk is based, it is desirable to review work in which the open Carius (wet digestion) method, followed by the Volhard titration, has been used; such a procedure has been used successfully and is advocated principally by foreign workers (6) (3) (15). It was proposed by von Korányi (31) for use in physiological fluids, and has been shown to yield quantitative results when used in milk (36) (2) (32) and other dairy products (25) (13). In the wet digestion method, the sample is digested by boiling in nitric acid and potassium permanganate or persulfate (3), in the presence of a known amount of silver nitrate, more than sufficient to combine with all of the chloride. The chloride combines with silver and the amount of excess silver is determined by titration, in the presence of ferric alum indicator, with a standardized solution of potassium sulfocyanate. The endpoint color changes from pure white to blood red and is comparatively distinct and easily read.

The applicability of the Volhard method is based on the fact that sulfocyanate has a greater affinity for silver than it has for iron, and combines with all of the excess silver to form a white precipitate of silver sulfocyanate. When the silver is completely used, the sulfocyanate combines with ferric alum indicator to form ferric sulfocyanate, which is soluble and has a distinct blood red color. With nitric acid present, interference from the presence of phosphate or other salts which precipitate silver in neutral but not in acid solutions is eliminated; moreover, there is no interference caused by copper, iron, and other common metals, as there is with chromate in Mohr's method.

Van Slyke and Donleavy (29) precipitated protein and chloride in plasma with silver nitrate, nitric acid, and picric acid. The material was then filtered and titration was carried out in an aliquot of the filtrate with standardized potassium iodide in the presence of an indicator containing starch, nitrite, and citrate. This procedure, slightly modified, was used recently by Reder (17) for determining chloride in milk. However, it has been shown by Van Slyke (28) and his co-workers that the Van Slyke-Donleavy method when applied to whole blood gave data which were 30 to 40 per cent too high, and they indicated that some component of the mixture other than chloride has the property of binding silver. They showed that in the case of whole blood, as is described below by the writer in the case of milk, the silver nitrate must be added after, rather than before, filtration. Van Slyke discarded the protein precipitation and potassium iodide titration method in favor of the wet digestion and Volhard titration method, and confirmed the precision of the latter.

Sunderman and Williams (27) found that, in blood analyses, the wet digestion and Volhard titration method is quantitatively accurate in comparison with standard gravimetric procedures. In analyzing solid tissues, however, they obtained concordant values for chloride only when they subjected the material to a preliminary alkaline digestion to prevent volatilization of chloride.

Conditions necessary for an accurate Volhard titration have been prescribed by Harvey (9) and by Whitehorn (34); they showed that it is not necessary to filter off the silver chloride precipitate before titrating, and that an essential consideration is the use of the proper amount of nitric acid, which decolorizes the untitrated indicator and also causes a maximum coagulation of the silver chloride crystals, with a consequent reduction of the amount of surface exposed for reaction with sulfocyanate. In addition, they showed that the presence of an abundance of ferric alum indicator retards any possible reaction between the precipitated silver chloride and the sulfocyanate and results in a deepening of the endpoint color. Whitehorn found further that accuracy was not increased by dilution, but that the volume of liquid should be small in order to insure sharpness of endpoint.

Numerous workers have used protein precipitation and filtration methods for the preparation of milk sera in which to determine chloride values. Poetschke (16) precipitated the proteins by means of copper sulfate and sodium hydroxide, filtered, and determined chloride in an aliquot by the Volhard method. Weiss (33) used aluminum sulfate and sodium hydroxide as the precipitant and titrated chloride in the filtrate with chromate indicator; Blood and Rowlands (2) used picric acid, and Drost (7) used nitric acid as the precipitant. The writer has used the method of Weiss in comparison with other methods and found that it gives lower and more accurate values than are obtained by Mohr's direct titration method. However, there is some error in protein precipitation methods if correction is not made for volume of precipitate (16) (6), and the work of making the material up to volume and filtering makes these methods so laborious that it did not seem advisable to include them in this study.

The dry ashing method is too laborious for routine or general laboratory work. Moreover, it has been indicated by numerous workers (3) (7) (5) that there is a considerable loss of chloride in the ashing method unless a base is added to prevent its volatilization (32) (36), and there is some evidence that a slight loss of chloride may occur even when compounds containing sodium are added (5) (3). According to H. E. Davies (5), the loss may occur mostly during the early stages of heating, and, in the absence of a sufficient amount of base, chloride passes off as hydrochloric acid.

In the official method of the Association of Official Agricultural Chemists (1) for determining ash in milk, nitric acid is added to aid in oxidizing organic material, and no mention is made of the addition of a base to bind the negative elements such as chlorine and phosphorus. It is evident, in view of the results of H. E. Davies mentioned above, and in view of the loss of chloride in ashing milk reported by Poetschke (16) and others, that the official method is deficient in these two respects—acid should not be added, and a basic material should be incorporated in the material to be ashed.

METHODS

1. Volhard titration without preliminary digestion: To 10 cc. of milk is added a measured quantity of the 0.0291 *N* special silver nitrate solution described below, more than sufficient to combine with all of the chloride; ordinarily 15 cc. is the amount used. The mixture is titrated with a 0.0291 *N* solution of potassium sulfocyanate. The number of cc. of silver nitrate solution used minus the titration value, multiplied by 0.01, equals the percentage of chloride.

Special silver nitrate solution: Exactly 4.9438 grams of C. P. Silver nitrate is dissolved in water, about 200 cc. of concentrated nitric acid and about 300 cc. of a saturated solution of ferric ammonium sulfate indicator are added, and the mixture is cooled and made up to 1 liter. C. P. silver nitrate can be used with reasonable accuracy as a primary standard, and the sulfocyanate should be standardized against it before use.

2. Open Carius (wet) digestion and Volhard titration: To 10 cc. of milk in a 300 or 500 cc. Erlenmeyer flack are added, first, 15 cc. of a 0.0291 *N* (4.9438 grams in 1 liter) solution of silver nitrate, or an amount more than sufficient to combine with all of the chloride, and then 15 to 20 cc. of concentrated nitric acid and about 50 cc. of water. The material is heated to boiling and 2 successive 5-cc. portions of a saturated solution of potassium permanganate are added slowly, drop by drop, to aid in digestion. The mixture is boiled (b. p. about 105–109° C.) slowly until it is clear yellow and contains no sediment other than coagulated silver chloride. It is then cooled, 5 cc. of a saturated solution of ferric ammonium sulfate indicator is added, and titration of excess silver is carried out with a 0.0291 *N* (2.8280 grams in 1 liter) solution of potassium sulfocyanate, until a trace of blood red color remains for at least 1 minute. Chloride is calculated as in method No. 1.

The 0.0291 *N* solutions described are of such concentration that each cc. equals 0.01032 gram of chlorine; the percentage of chloride may be read directly, in analysis of 10 cc. of milk and with solutions of this normality, if one assumes that the specific gravity of the milk is 1.032. In the early part of the work described herein, 0.1711 *N* solutions were used, for the reason that, with solutions of this concentration, direct readings for sodium chloride were made in analyzing weighed samples of cheese in a method proposed by the writer and published previously (25) (35). With the higher concentration of reagents, 15-cc. samples of milk were used and the correction factor used was 0.0392. If 0.10 *N* solutions are used with 10-cc. samples, the factor is 0.03436; with 10-gram samples it is 0.03546.

3. Direct titration with silver nitrate: In titrating with potassium chromate indicator, 10 cc. of milk was measured accurately into a porcelain dish and 50 cc. of water and 1 cc. of a 10 per cent solution of potassium chromate were added. The titration was then carried out with a 0.0291 *N* solution of silver nitrate until a definitely visible brick-red color became evident.

In titrating with dichlorofluorescein indicator, the procedure outlined above was used, except that 5 drops of indicaor was added; the titration was carried out until a faint pink tint was evident, as described by Hastings and Beach. The indicator was made up by dissolving 0.1 gram of the reagent in 100 cc. of 70 per cent ethyl alcohol.

4. Dry ashing: Ten cc. of milk was measured accurately into a platinum crucible, dried over night under vacuum at 100°, and ashed in a muffle at a very low red heat, at not over 600°; in some cases the samples were ashed in porcelain dishes over a free flame, and in some cases it was necessary to remove the charred material by washing it with water onto a filter and complete the ashing of the filter paper and its contents. The method is essentially the same as that of the Association of Official Agricultural Chemists (1) except that it was found necessary to add a base to prevent loss of chloride, and the use of nitric acid as an oxidant was dispensed with; the base used was sodium hydroxide, 5 cc. of a normal solution being added to the sample before it was dried.

RESULTS

In the present work, the Volhard method has been taken as a standard for quantitative accuracy for the reason that, among all the references which the writer has found in which it has been used in comparison with other methods and in which the conditions prescribed by Volhard and by others mentioned herein have been adhered to, the accuracy of results obtained has not been questioned.

Data obtained in analyses of several milk samples by the methods outlined above, and representative of a large number of comparative tests, are shown in Table 1. The preliminary alkaline digestion method of Sunderman and Williams, referred to above, was used with the Volhard titration

TABLE 1

Percentage of chloride in milk samples as determined by different methods

Sample	1 Volhard titration without digestion	2 Open Carius digestion and Volhard titration		3 Direct titration with silver nitrate		4 Ashing and Volhard titration	
		A	B	Potassium chromate indicator	Dichloro-fluorescein indicator	Without added base	With sodium hydroxide
Right front (mastitis)	0.310	0.306	0.309	0.346	0.340	0.276	0.300
Right rear (normal)	.092	.090	.092	.130	.110	.055	.089
Left front (normal)	.110	.110	.110	.141	.129	.080	.104
Left rear (mastitis)	.208	.206	.206	.250	.238	.170	.202
Mixed herd milk	.079	.076	.078	.111	.103	.060	.075
Same plus 0.05% Cl.	.128	.128	.128	.160	.150	.105	.124
Milk cont'g. 2.1% casein	.085	.083	.083	.110	.105	.075	.080
Milk cont'g. 2.7% casein	.090	.089	.088	.135	.120	.070	.088

[A] **Open Carius (wet) digestion with HNO_3, $KMnO_4$,** and an excess of $AgNO_3$, followed by titration with KCNS, with ferric alum indicator.
[B] **Preliminary alkaline digestion** followed by open Carius method.

as an added precaution against the volatilization of chloride in digesting the samples for which data are shown under method 2B. The concordance of these results with results obtained by the ordinary open Carius (wet digestion) method followed by the Volhard titration, shown under method 2A, shows that there is no loss of chloride in the ordinary wet digestion method. It was found, however, that as Collier (4) and others (32) have pointed out, a considerable loss of chloride occurred when milk was boiled with nitric acid without the previous addition of an excess of silver nitrate. It was noted also that there was no difference in results whether or not the silver chloride precipitate was first removed by filtration before titration.

Results obtained by the Volhard titration in undigested samples by the use of the combined silver-acid-alum reagent (method No. 1), shown in Table 1, were consistently concordant with results obtained by the open Carius (wet digestion) method taken as a standard.

The Volhard titration, conducted with the combined reagent and in undigested milk, is characterized not only by accuracy but also by ease and speed of operation; once the two standardized solutions have been prepared, the test is carried out nearly as quickly as is the direct titration with chromate and silver nitrate. As a result of the accuracy with which the Volhard titration was conducted in undigested samples with the combined silver-acid-alum reagent, the writer recommends this procedure—method No. 1 described above—for determining chloride in milk, in cases in which accuracy is sufficiently important to justify the slight amount of extra time which may be required in comparison with the direct Mohr mehod.

In the titration, a definite change in endpoint color is apparent when 3 to 5 extra drops of titrating solution are added, equal to 0.15 to 0.25 cc., which amount is equivalent to 0.0015 to 0.0025 per cent chloride. The titration values were about 0.1 cc. higher when samples being titrated were diluted with 50 cc. of water in comparison with undiluted samples—an amount equivalent to 0.001 per cent chloride. Under the conditions specified, endpoint errors do not exceed 0.3 cc. in titration value, or 0.003 per cent chloride.

Results obtained with chromate indicator in a large number of tests were usually 0.02 to 0.045 per cent higher than those obtained by the Volhard titration; results with dichlorofluorescein were about midway between the normal values for the Volhard titration and the abnormally high values obtained with chromate. Similar results were reported by Hastings and Beach. It is shown in the lower two lines in Table 1 that the positive error for both chromate and dichlorofluorescein is increased when the percentage of casein in the sample is high. This fact indicates that the subtraction of a constant correction factor would lead to erroneously variable results.

Attempts were made to increase the accuracy of the direct titration by analyzing samples prepared as follows: (a) Proteins were precipitated by adding to the undiluted sample an equal volume of a saturated solution of zinc sulfate in 70 per cent ethyl alcohol; (b) Casein was removed by adding rennet and filtering, and an aliquot was titrated; (c) Samples were titrated without being diluted with water. Data secured in these tests are shown in Table 2.

TABLE 2

Effects of dilution of sample, precipitation of protein, and removal of casein, in the direct titration of chloride in milk

Sample	Volhard titration	Direct titration with silver nitrate			
		Potassium chromate indicator		Dichlorofluorescein indicator	
		undiluted	with 50 cc. water	undiluted	with 50 cc. water
	%	%	%	%	%
Mixed herd milk	0.081	0.107	0.120	0.098	0.110
Same, protein precipitated[1]		.098	..	.093	..
Whey from above milk (casein removed)	.083	.097	.109	.093	.105

[1] Ten cc. of a saturated solution of zinc sulfate in 70 per cent ethyl alcohol was added to 10 cc. of milk, as a protein precipitant, before titration.

The results indicate that, in the case of either indicator, chloride values can be reduced slightly by titrating the samples undiluted rather than diluted with water, and by precipitating protein or removing casein. While these modifications add to the accuracy of the method, the values are still considered to be too high and the endpoints too indistinct in the case of both indicators.

The fact that chemical combination occurs between silver and milk protein, as claimed by W. L. Davies in work cited above, was demonstrated by analyzing samples of milk serum prepared as follows:

(A) To 20 cc. of milk in a 100 cc. volumetric flask there were added 50 cc. of water and 10 cc. of nitric acid, the flask was filled to the 100 cc. mark with water, the contents were mixed, and the material was filtered. To 50 cc. of the filtrate there was added 15 cc. of 0.0291 *N* silver nitrate solution and the Volhard titration was then carried out; (B) Thirty cc. of 0.0291 *N* silver nitrate solution and 10 cc. of ferric alum indicator were added to 20 cc. of milk and after the sample was made up to volume and filtered as described above, 5 cc. of nitric acid was added to 50 cc. of the filtrate and the Volhard titration was carried out. The titration values in the filtrates were 7.00 cc. and 5.20 cc. of 0.0291 *N* sulfocyanate, respectively. It was apparent that a portion of the silver had combined with the protein precipitate in the second sample, resuling in a low value for silver in the filtrate and a positive error for chloride in the analysis. The error is similar to that found by Van Slyke in blood analyses. The combination of silver with protein indicates that, if a filtration method is used, the silver nitrate should be added to the serum after filtration rather than to the milk before filtration. It is likewise a verification of the belief, expressed by W. L. Davies, that the positive errors obtained in direct titrations with silver nitrate are caused partially by the tendency of silver ions to combine with protein. The validity of this conclusion is strengthened by the fact, shown in Table 1, that errors in direct titrations are greatest in those samples which contain the most protein. In the Volhard titration of undigested milk, the results indicate that the affinity of silver for sulfocyanate is sufficiently greater than that for milk protein to overcome this difficulty.

It was noted that when a pure solution of sodium chloride was titrated with a pure solution of silver nitrate of the same normality, with chromate indicator, a slight excess of silver nitrate solution was always required to produce a visible endpoint; the average of the excess titrations, with 0.0291 *N* solutions, was 0.15 cc.; similar results were reported by Mohr. This error, which did not occur when dichlorofluorescein was used, serves to point out the necessity of standardizing the silver nitrate solution against a pure solution of sodium chloride before use.

There was a considerable loss of chloride in the dry ashing method, as shown in Table 1,—a loss which varied markedly and which apparently was not materially greater in high-chloride than in low-chloride samples; there was some evidence of a slight loss even when a base was added to bind the chloride. Poetschke (16) recommends the use of sodium carbonate for this purpose, in an amount equal to 5 per cent of the organic matter present. In this work, however, it was found that less difficulty was encountered because of foaming, and less time was required to burn the charred material, if sodium hydroxide was substituted. Results of tests in several milks, in which varying amounts of sodium hydroxide were added to samples of each milk before ashing, showed that an addition of 5 cc. of normal, chloride-free solution to 10 cc. of milk was more than sufficient to produce a maximum retention of chloride.

SUMMARY

The direct titration of chloride in milk by modifications of Mohr's method, with silver nitrate and with potassium chromate indicator, yields results which are so erroneously high and variable that this method, even with a correction factor, is not considered suitable for the accurate determination of chloride in milk. The direct titration method with dichlorofluorescein indicator yields somewhat lower results, but results which are still considered too high and erratic to be relied upon. Indistinct endpoints with either indicator, and the adsorption of silver by protein, are pointed out as important sources of error.

An easy and quantitatively precise modification of the Volhard method is proposed for determining chloride in milk. One combined reagent, containing standardized silver nitrate, nitric acid, and ferric alum indicator, is added to the sample and the percentage of chloride is determined by titrating with standardized potassium sulfocyanate.

Sources of error and methods of improvement in other methods, as well as conditions necessary for accurate Volhard titrations, are described.

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THE PHOSPHATASE TEST. A REVIEW OF THE LITERATURE ON ITS APPLICATION FOR DETECTING IRREGULARITIES IN THE PASTEURIZATION OF MILK AND DAIRY PRODUCTS

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The need of a practical test for detecting the degree of pasteurization of milk has been felt for some time. Bacterial counts, recording thermometer charts, and plant inspections do not always furnish a reliable means for ascertaining that milk has been properly pasteurized.

It might be well to make brief mention here of the amylase test which was the first such test intended to aid in detecting irregularities in the pasteurization of milk. It was proposed by Rothenfusser (1) in 1930. Gould (2) in 1932 suggested a modification, and Leahy (3) in 1934 reported another modification and acclaimed it of great value in detecting improperly pasteurized milk. The amylase test did have some merit, but was not accurate or sensitive enough for detecting minor variations in pasteurizing treatments, as reported by Gilcreas and Davis (4) and Storrs and Burgwald (5).

In 1933 Kay and Graham (6) found, "that phosphatase is sufficiently thermolabile to be destroyed completely by pasteurization if this process is properly carried out."

They described a qualitative method for distinguishing between raw and pasteurized milk or cream and between butters made from raw and pasteurized cream.

This was the start of the "phosphatase test" as we know it today. It has, of course, been considerably modified and improved.

FACTS ABOUT THE ENZYME PHOSPHATASE

All raw milk contains the enzyme phosphatase (6, 7, 19). Milk from individual cows varies in the amount of phosphatase present (7, 8). Milk from cows in early stages of lactation has low phosphatase content (7, 42). Milk from abnormal (mastitis) udders usually has higher phosphatase content than milk from normal udders (8, 43, 44). It is not likely to vary greatly for mixed herd milk. That the phosphatase enzyme is associated with the fat is shown by Kay and Graham (6). It is not fat soluble, however, but is present in the thin protein layer which covers the globules or is adsorbed to the fat globule in such a way that the greater part is removed in the buttermilk.

There is no relation between fat content and phosphatase content of raw milk, nor is there any relationship between the phosphatase content of the raw milk and of the phenol values obtained on the same milk pasteurized (5).

The fact that the phosphatase is associated with the fat globules requires that the fat be well mixed with the milk before testing.

DESTRUCTION OF PHOSPHATASE VS. PATHOGENIC ORGANISMS

Kay and Graham (6) show that at any temperature from 60 to 75° C. (140–167° F.) wherein 96% of the phosphatase is destroyed, there is a complete destruction of B. tuberculosis.

Kay and Neave (19) report that over all ranges of temperature and time, *Mycobacterium tuberculosis* is destroyed rather more quickly than the phosphatase, hence, all common pathogenic organisms which may be present in milk would be destroyed.

As all raw milk contains the enzymes phosphatase (6, 7, 19) then we may assume that any milk which has been heated to a temperature above 60° C. for a sufficient length of time to destroy 96% or more of the phosphatase present would be free of pathogenic organisms providing the milk had not become contaminated with them after pasteurization. This would mean clean, treated equipment and containers, together with medical inspection of employees to prevent pathogens getting into the milk after pasteurization.

Kay (20) reports that in the "flash" as well as in the "holder" range of temperatures for pasteurization, phosphatase is somewhat less readily destroyed than is *Mycobacterium tuberculosis.*

KAY AND GRAHAM TEST

In 1935 Kay and Graham (7) reported the phosphatase test for pasteurized milk. This test used disodium phenyl phosphate for the phosphoric ester as substrate in place of the sodium B-glycerophosphate reported in (6). This new substrate is hydrolyzed faster by the phosphatase than is the glycerophosphate. Also, in this new test they measured the free phenol liberated by the enzyme rather than the inorganic phosphate liberated.

The principal steps in conducting the test are:—1. the addition of small portions of milk under test to a large excess of phosphoric ester; 2. incubating the milk phosphoric ester mixture under standard conditions during which time the phosphatase if present hydrolyses the ester (which otherwise is quite stable); 3. stopping the action after a given period of time followed by the colorimetric determination of the end products.

Using a Lovibond tintometer for measuring the color after a 24 hour incubation period, they set a limit of 2.3 Lovibond blue units as a standard, as no sample which had been properly pasteurized at 145° F. for 30 minutes gave a color exceeding this limit. Storrs and Burgwald (5) and Burgwald and Giberson (15) found 2.3 Lovibond blue units a satisfactory limit for milk pasteurized at 143° F. for 30 minutes using Kay and Graham's method with 24 hour incubation period.

Kay and Graham report that this test would detect milk heated at 145° F. for 20 minutes instead of 30 minutes or milk heated to 1.5° F. below the minimum temperature for pasteurization, or the admixture of 0.25 per cent raw milk to properly pasteurized milk.

GILCREAS AND DAVIS MODIFICATION

Results of work with this test in the United States were first reported in 1936 by Gilcreas and Davis (4) using Kay and Graham's (7) procedure, modified only in the method of reading, by preparing color standards containing known amounts of phenol instead of a Lovibond tintometer. As a result of their study using a 24 hour incubation period, they reported that a phenol value of 0.037 mg. or less per 0.5 ml. of milk indicated adequate pasteurization (143° F. for 30 minutes). They reported that the test would detect as little as 5 minutes variation in holding time, additions of as little as 0.1 per cent raw milk to properly pasteurized milk, and could easily detect variations in temperature. They reported correctly interpreting the treatment of 94 out of 97 samples by the use of this test. (Prepared samples, treatment unknown to those making tests.)

In 1938 Gilcreas (26) reported the results obtained by eleven cooperating laboratories in testing six weekly series of twelve samples of milk and cream, using the Gilcreas and Davis modification of the Kay and Graham method. He increased the standard phenol value to 0.05 mg. of phenol per 0.5 ml. of milk as indicative of properly pasteurized milk at 143° F. He explains that "further experience indicated that variations in time of cooling or preheating might cause some variations in this value, so $<$ 0.05 mg. phenol per 0.5 ml. milk was selected in this evaluation as an indication of complete treatment at this temperature."

Using this standard he found that the cooperating laboratories had reported 104 samples out of 110 that had been heated to 143° F. for 30 minutes had a phenol value of less than 0.05 mg. per 0.5 ml. of milk. Therefore, pasteurized milk was detected with a precision of 96 per cent. Addition of 0.1 per cent raw milk was detected with a precision of 50 per cent while larger amounts were detected without exception. Holding at 143° F. for 25 minutes was not detected with the precision expected. Gilcreas thought this was probably due to variations in preheating time. Twenty minute holding or less was detected without exception.

Burgwald and Giberson (15) found that 0.04 mg. of phenol per 0.5 ml. of milk was satisfactory as a standard for milk pasteurized at 143° F. for 30 minutes and enabled one to more accurately detect minor discrepancies in pasteurization. (Certainly a standard of 0.05 mg. phenol per 0.5 ml. of milk is generous and should never result in an interpretation of properly pasteurized milk as being underpasteurized.)

SCHARER (NEW YORK) LABORATORY TEST

In 1937 (published in 1938) Scharer (10) reported a modification of the phosphatase test which was shorter, requiring only one hour incubation in place of 24, and less expensive than Kay and Graham's method. Scharer used 2.6 dibromoquinonechloroimide (BQC) in place of Folin and Ciocalteu reagent. The sensitivity of BQC to phenol is one part in twenty million and has the further advantage of reacting only with those phenols in which the position part of the hydroxyl group is unsubstituted to form indophenols. Folin and Ciocalteu's reagent is lacking in specificity and has the disadvantage of reacting with other substances, such as tin salts, ferrous iron, tyrosine and tryptophane (11, 12). However, these substances should not be present in dairy products to the extent that they would effect the test. Their presence, however, would be detected in the controls.

Scharer used phenol color standards and set as a standard for properly pasteurized milk (143° F. for 30 minutes) the blue color developed by 0.5 p.p.m. (2.5 gamma) of phenol in 5 ml. or 2.5 phosphomonoesterase units. A color greater than this would indicate faulty pasteurization.

He proposed for convenience to identify as a unit of blue the amount of color produced by 0.001 mg. of phenol per 5 ml. solution. Thus, the standard containing 0.5 p.p.m. of phenol would contain 0.0025 mg. (2.5 gamma) phenol per 5 ml. or 2.5 units, the standard containing 1.0 p.p.m. would contain 0.005 (5 gamma) per 5 ml. or 5 units, etc.

He reports the test will detect 1° F. variation in temperature, 25 minutes heating at 143° F. in place of 30 minutes, and the addition of 0.5 per cent of raw milk to properly pasteurized milk or cream. He later reported that 0.1 per cent of added raw milk could be detected, and that for greater sensitivity a two hour incubation period should be employed (28).

New inorganic color standards to replace this series of phenol standards because of their comparative instability was reported by Scharer in 1939 (28).

Burgwald and Giberson (15) found that 2.5 phosphomonoesterase units (0.5 p.p.m. of phenol) were too low to indicate properly pasteurized milk by the holder method (143° F. for 30 minutes). It was found by the use of a Cenco photelometer that the blue color developed by milk pasteurized at 143° F. for 30 minutes equivalent to from 0.7 to 0.9 p.p.m phenol. Tracy and Hahn (21) found that milk pasteurized at 143° F. for 30 minutes developed a blue color equivalent to 0.6 p.p.m. of phenol, and Hahn and Tracy (22) found that milk pasteurized at 143° F. for 30 minutes developed a blue color equivalent to 0.8 p.p.m. of phenol. Both of these tests were obtained with the use of a photoelectric cell for measuring the blue color.

It is possible that the use of the new color standards will prove more satisfactory than the old ones.

SCHARER'S RAPID FIELD TEST

Scharer (10) also developed a rapid test for use in the field. This field test could be completed in 10 to 15 minutes at the plant. (A compact kit containing all necessary equipment and chemicals is obtainable from R. P. Cargille Company, New York.) This test was not as sensitive as the laboratory test. Properly pasteurized cream gave a grey or white color, and properly pasteurized milk a grey or brown color. According to Scharer, samples that developed 10 or more units by the laboratory test would also give a blue color with the field test. In some cases on cream it gave a blue color when the laboratory test showed 7.5 units of color.

Scharer (13) later reported improvements in his rapid field test. The new test was run in the usual manner, but after development of color, 2 ml. of normal butyl alcohol (neutral) are added and the indophenol formed is extracted by inverting the test tubes slowly at least 10 times for milk, four or five times is usually sufficient for cream, further extraction usually extracts the fat adding yellow and green color to the alcohol layer. The appearance of any blue or blue green color in the alcohol layer is indicative of improper pasteurization.

Inorganic color standards have been prepared for indicating the maximum color value permissible for pasteurization at 142° F. for 30 minutes. This is reported to be the same color obtained when 0.2 per cent raw milk is added to properly pasteurized milk. A color standard has also been prepared for 0.5 per cent added raw milk.

The use of this modification, together with the following improvements in reagents have resulted in a more accurate test. Improvements have been made in preparing commercially a more stable, practically phenol free ester carefully controlled as to pH and containing an almost negligible amount of Na_2HPO_4. This ester is so pure that Scharer reports that a 50 per cent reduction in the concentration of the substrate is feasibe. (Available under name of Phenfree.)

BQC reagent has been improved and made purer, reacts more quickly, and gives better results. The catalytic effect of magnesium has been investigated and found to result in a further gain in hydrolysis of the substrate per unit of enzyme. All of these improvements together have resulted in making the rapid field test comparable to the one hour laboratory test and the 24 hour Kay and Graham test in accuracy, according to Scharer.

Giberson (14), Burgwald and Giberson (15) found this improved field test to be nearly as effective as any of the laboratory tests. They found the BQC varied in sensitivity, however. (This, however, has been corrected since their work was reported.) BQC tablets now obtainable have proven more sensitive and reliable. Instructions concerning precautions necessary in making tests should be followed closely.

Roger (16, 17) found the test to be very effective in detecting discrepancies in pasteurization. The fact that it can be run in the plant at the time of taking samples, aides materially in tracing the trouble when underpasteurized milk is found.

He reports that while amyl alcohol does not extract as much of the indophenol blue it has some advantage over butyl alcohol in that it does not extract as much of the yellow color from high color milk and cream, which may interfere with tests on slightly underpasteurized products. However, butyl alcohol is satisfactory for most practical purposes. He, also, reports that incubating the samples for 30 minutes in place of 10 minutes develops more blue color in underpasteurized samples, but has no effect on properly pasteurized samples, thus giving greater color contrast between properly pasteurized samples and samples which may be only slightly underpasteurized.

By this method he found that milk which was heated to 142° F. or lower for 40 minutes or less gave a blue color. Also milk heated to 143° F. for 25 minutes or less gave a blue color and milk heated to 144° F. for 20 minutes or less gave a blue color. Milk heated to 145° F. for 20 minutes or longer gave a color indicative of properly pasteurized milk. Incubation at 47° C. for 30 minutes had little or no effect on the test when compared to 37° C. for 30 minutes. He reports, some 18,000 tests run during the past year have proven the Scharer rapid field test to be reliable when properly used.

A report given by Gilcreas (27) at the meeting of the Association of Official Agricultural Chemists, Washington, D. C., November 15, 1938, showed that of the various laboratories cooperating in making tests on samples sent out by him, no laboratory correctly determined the treatment of all samples. Some of them found properly pasteurized samples as being underpasteurized and underpasteurized samples as properly pasteurized. The total results of this test showed it to be very ineffective. The Department of Dairy Technology, Ohio State University, was one of the cooperating laboratories and a comparative test using Kay and Graham's method was also run on 60 of the 72 samples submitted. The results of this comparison were reported by Burgwald and Giberson (15). At that time the key to the samples was not available. Since obtaining the key it was found that 13 of the 60 samples had been properly pasteurized and that they were so indicated by all of the tests. Of the 47 samples which had been underpasteurized 44 had been detected by the Kay and Graham test, and 25 by the improved field test and only 7 by the old field test. This was when using the buffer substrate and BQC tablets for the field test sent out by Gilcreas. When reagents prepared in the laboratory were compared with those received for the test, it was found that there was a decided difference in the sensitivity of the BQC solution made up from different tablets and from that prepared in the laboratory from BQC powder. We have since obtained tablets that have proven very uniform as to sensitivity and stability. Using the more sensitive BQC solution we found that 35 samples were underpasteurized. Six other samples showed a slight greenish tint which had been interpreted as pasteurized but which should have been interpreted as underpasteurized. In no instance were any samples which had been properly pasteurized interpreted as underpasturized.

It is difficult to understand in Gilcreas' (27) report how 42 out of 242 tests on 16 samples of milk and cream pasteurized at 143 and 145° F. for 30 minutes should be interpreted as underpasteurized by this test. Certainly it looks as though reagents or glassware had been contaminated as no other investigator had ever reported properly pasteurized milk as underpasteurized by this test (10, 13, 14, 15, 16, 17). No doubt the yellow color extracted by the butyl alcohol interfered in some cases, especially with cream.

It should be stated, however, that the majority of the laboratories reported those samples which had been properly pasteurized, as such.

Scharer (38) and Rice (34) report that the test has been applied to more than 100,000 samples by the New York City Department of Health and has been proven satisfactory. Certainly Scharer's and Roger's results should merit consideration, in view of the large number of samples involved.

Roger's modification (16, 17) using amyl alcohol in place of butyl, and a 30 minute incubation time in place of ten minutes should give more accurate results.

FACTORS WHICH MAY EFFECT THE ACCURACY OF THE PHOSPHATASE TESTS, AND PRECAUTIONS NECESSARY

Anderson, Herschdorfer and Neave (12) made a careful study of Kay and Graham's (7) technic and reported as follows:

Stability of Reagents

Buffer substrate.—This solution with a few drops of chloroform to prevent growth of microorganisms if kept in a refrigerator will show no hydrolysis for at least nine weeks.

Folin's reagent must be entirely free of greenish tinge. It showed no sign of decomposition in four months. The dilute reagent did not show any sign of decomposition over a period of one week. Kay and Graham (7) report it must be carefully made up according to directions. Sodium carbonate solution had a tendency to form a precipitate after a short time. It is advisable to control its strength by titration. It is also advisable to add a few drops of chloroform.

Equipment

Accurate pipettes for measuring milk and carbonate solution must be used.

Test tubes marked at 10 ml. must be accurate within ± 0.1 ml.

Filter papers—Whatman No. 30 filter papers are satisfactory but No. 42 papers give more satisfactory results. Kay and Graham (7) report a good acid washed paper should be used. Whatman No. 30 was satisfactory.

Methods.

All glassware used should be washed with hot soda solution and rinsed with distilled water.

Variations of ½ hour in incubation period had no effect on results.

Gilcreas and Davis (18) showed that there was no significant variation in phenol values if samples are incubated between 18–24 hours but progressive inaccuracies occur when a period shorter than 18 hours is used.

After incubation the tubes should be cooled below 20° C. before adding

Folin reagent, as there is a tendency to increase slightly the blue color at higher temperatures.

Slight variations in quantity of Folin reagent added had no effect.

It is important to filter at end of three minutes after adding the Folin reagent, and to remove the funnels as soon as 10 ml. of filtrate has been obtained. Delay will give a higher reading (more blue color).

The exact amount 2.0 ml. of sodium carbonate of the proper strength (14%) should be used. Too little or too low concentration will give high results; too much or too strong will give low results.

All tests should be run in duplicate and results should agree within 0.1 Lovibond blue unit.

The control tests are completed immediately. Kay and Graham (7) report that the control tests should not develop more than 1.5 Lovibond blue units.

Kay and Graham (7) caution that care must be exercised to keep phenols, etc., at a safe distance from test reagents and apparatus in the laboratory.

Scharer (10) reported that disodium phenyl phosphate may contain free phenol and should be washed with ethyl ether until the washings give a negative test for phenol. Then air dry briefly to remove ether and then dry in desiccator, and store in refrigerator.

The buffer substrate should have a pH of 9.6 and should be checked, using Thymolphthalein as an indicator.

Scharer (10) reports that certain precautions should be observed in running his laboratory test.

A blank should be run on reagents daily. If the blank produces blue color, the solution of buffer substrate should be discarded, or it may be used if phenol is extracted with butyl alcohol (13, 16). Samples of milk and cream, if cold, should be warmed to room temperature before pipetting. If incubator, rather than water bath is used, the test tubes should be placed in water at 40° C. for a minute or two before placing in the incubator (10). Avoid phenolic resin bottle closures (13).

If filtrate is clouded following use of basic lead acetate, or on addition of ¼ cc. borate buffer, it may be clarified by centrifuging at a speed of 800 r.p.m. He later states that the turbidity of filtrate can be cleared up by adding a few drops of m/5 Na_4PO_7 solution (13). Precautions must be taken to prevent saliva getting into test.

Burgwald and Giberson (15) found that BQC powder was more sensitive than the tablets. Scharer (13, 28) reports that both substrate and BQC tablets have been improved. BQC solution should be stored in brown bottles and best not to use when over 12 hours old.

In Scharer's rapid field test, technic must be followed closely, and published precautions strictly adhered to.

EFFECT OF AGE OF SAMPLE AND BACTERIA ON THE PHOSPHATASE TEST

Age has no apparent effect on the phosphatase test when samples are held under refrigeration so that there is no appreciable growth of bacteria and samples do not sour (4, 10, 12, 15, 18). Samples have been held up to 15 days in some cases, without effect on test. When samples soured Gilcreas and Davis (4) reported samples of pasteurized reacted as underpasteurized. They later report that increased acidity lowered the phenol values (18). Scharer (10) found that on souring most samples gave a lower phenol value, and none gave a higher value with his test. When sour samples were neutralized with Na_2CO_3 to a pH of 6.6 before sampling phenol values again approached the original as obtained on the fresh milk. Burgwald and Giberson (15) found the same to be true.

Some types of bacteria do effect the Kay and Graham test (7, 12, 15) and this may account for the fact that Gilcreas and Davis (4) obtained an increase in phenol value when properly pasteurized milk soured; due probably to the presence of proteolytic organisms (15). Folin and Ciocalteu (11) show that certain protein decomposition products such as tyrosine and tryptophane will give a blue color with their reagent. Scharer (10) states that these products would not react with BQC.

The writer has since tested boiled milk inoculated with S. liquefaciens and incubated and obtained a positive test by the Kay and Graham method but a negative test by Scharer's method.

Even though certain bacteria may cause interfering products to be formed in milk, it would be rare that they would be present in sufficient numbers to cause errors in the Kay and Graham test. However, if they should be present to such an extent, the use of control tests would indicate it.

When refrigeration is not available and it is necessary to hold samples for some time or to ship them, they may be preserved without effect on the test. Tracy and Hahn (21, 22) found that corrosive sublimate tablets could be used as a preservative. Warming the samples to 100° F. so that the fat could be remixed thoroughly had no effect on the test.

COMPARISON OF THE VARIOUS PHOSPHATASE TESTS FOR ACCURACY IN DETECTING DISCREPANCIES IN THE EFFICIENCY OF PASTEURIZATION OF MILK

Burgwald and Giberson (15) compared the various laboratory tests on both laboratory pasteurized samples and on commercially pasteurized samples of milk.

Using 2.3 Lovibond blue units as a standard for the Kay and Graham test, the samples of milk pasteurized in the laboratory at 143° F. for 30 minutes showed 2.5 blue units. However, only a 3 minute preheating period was used. All underpasteurized samples were detected as such. On commercially pasteurized samples, temperatures and holding time checked. (Preheating period of 18 to 20 minutes.) Three samples of properly pasteurized milk out of 29 were indicated as underpasteurized, while 26 out of 26 actually underpasteurized were detected as such.

Using Gilcreas and Davis standard of 0.04 mg. phenol or less per 0.5 ml. of milk as indicating properly pasteurized milk, all samples of laboratory pasteurized milk tested were properly identified except one heated to 142° F. for 40 minutes which was indicated as properly pasteurized. Of the plant pasturized samples no properly pasteurized sample was indicated as underpasteurized, but four out of 26 were indicated as properly pasteurized which had been underpasteurized. Three of these samples, however, had been only slightly underpasteurized and one had had 0.1 per cent raw milk added to milk pasteurized at 143.5° F. for 30 minutes.

Scharer's laboratory test using 2.5 phosphomonoesterase units as indicating proper pasteurization was found to be somewhat too low. (Also shown in reference 21, 22.) All samples of underpasteurized milk were detected with this unit, but too many properly pasteurized milks were indicated as underpasteurized as follows, two laboratory pasteurized samples and 11 out of 29 plant pasteurized samples were so indicated.

When less than 5.0 units were used as an indication of properly pasteurized milk, all laboratory pasteurized samples were properly identified and all plant pasteurized, with the exception of one, were properly identified. This exception was a sample of milk which had been pasteurized at 143° F. for 30 minutes to which 0.1 per cent raw had been added.

The fact that these results do not agree with the standard set by Scharer (10) seem to be due to a possible difference in the sensitivity of the BQC solutions used. Tracy and Hahn (21, 22) also found 2.5 units to be too low. Scharer (13) reports that BQC reagent has been improved, reacts more quickly and gives better results; this then, may be the reason for the differences.

When we compare all three tests critically, we find them to be of approximately equal accuracy in detecting underpasteurization. All of the tests detected milk which had been heated to only 142° F. for 30 minutes, milk which had been held for only 25 minutes or less at 143° F. and the addition of 0.1 per cent added raw milk.

The tests were equally effective on milk pasteurized in the plant by the high temperature short time holding method. A comparison was also made between the Scharer old rapid field test, the improved field test, and the Kay and Graham test.

The results show that the improved rapid field test using 10 minute incubation is much more sensitive than is the old one, but it is not quite as sensitive as the laboratory test.

APPLICATION OF PHOSPHATASE TEST FOR TEMPERATURE OTHER THAN 145° F. FOR 30 MINUTES

This test according to Kay and Graham (7) could be adapted or modified for any pasteurizing temperature by simply changing the incubation period of the test. They suggested the following incubation periods for the different temperatures. (Taken from their graphic presentation.)

Temperature of pasteurization 30 minute holding °*F*.	Approximate length of incubation period
140	1 hr. 0 min.
141	1 hr. 30 min.
142	2 hr. 30 min.
143	4 hr. 20 min.
144	7 hr. 45 min.
145	24 hr. 0 min.

Gilcreas and Davis (4) showed that a 24 hour incubation period could be used for a pasteurizing temperature of 143° F. for 30 minutes. They, however, used a different standard for measuring the color formed by the liberated phenol than did Kay and Graham. Gilcreas and Davis found the test to be somewhat more sensitive than did Kay and Graham.

Storrs (8) and Storrs and Burgwald (5) using Kay and Graham's (7) method with Lovibond tintometer and 2.5, 5.0, and 24 hours incubation periods on samples of milk pasteurized in laboratory, found that a 2.5 hour incubation was too long for milk pasteurized at 140° F. for 30 minutes. An incubation period of more than 2.5 hours but not more than 5 hours should be used for milk pasteurized at 142° F. for 30 minutes. An incubation period of five hours was too short to detect underpasteurization of milk at 143° F. for 30 minutes, and an incubation period of 24 hours was slightly too long in a few instances, however, the maximum blue color developed with a 24 hour incubation period was never more than 2.5 units. Another factor that must be considered here is that the preheating time was only three minutes while in commercial practice the preheating time is usually 15 to 20 minutes. They were unable to detect milk as underpasteurized which had been heated to 145° F. for 20 minutes with a 24 hour incubation period. This did not agree with the findings of Kay and Graham (7) who were able to detect 20 minute holding at 145° F.

Scharer (10) reported for his laboratory test that pasteurization at various temperatures for 30 minutes gave results expressed in this proposed phosphomonoesterase units as follows:

138° F.	250–500 units
140.5° F.	100 units
142° F.	10 units
143° F.	2.5 units

APPLICATION OF THE PHOSPHATASE TEST TO MILK PASTEURIZED AT HIGH TEMPERATURE FOR SHORT HOLDING PERIODS

The phosphatase test is equally as effective for detecting irregularities in high temperature short time holding systems of pasteurization as in the vat systems of pasteurization (5, 8, 9, 10, 12, 14, 15, 23, 24, 25).

Storrs and Burgwald (5) report that an incubation period of 24 hours was too long for milk pasteurized in the laboratory at 160° F. for 15 seconds. However, it was satisfactory for milk held for 20 seconds at that temperature. Burgwald and Giberson (15) showed that all of the tests run on commercially pasteurized samples of milk at 160.5° F. for 16 + seconds gave negative readings.

Most commercial plants using this system hold for slightly longer than 15 seconds. Scharer (10) found the length of holding time to be 17 to 18 seconds, Burgwald and Giberson (15) 16 + seconds, and Hoy and Neave (23) reported the holding time to be in the order of about 20 seconds.

APPLICATION OF THE PHOTELOMETER TO THE PHOSPHATASE TEST

Anderson, Herschdorfer and Neave (12) showed that either a photometer or colorimeter could be used for accurately measuring the blue color developed in the Kay and Graham phosphatase test. They reported that with a photometer it was possible to measure the color to an accuracy of 0.02 of a unit.

Tracy and Hahn (21, 22) used a photoelectric cell with the Scharer laboratory test. They report that the photelometer was more sensitive than the naked eye and was necessary to detect 0.1 per cent added raw milk; 0.2 per cent or more could readily be detected with the naked eye. With its use they detected 5 min. variation in holding time and 1.0° F. variation in temperature.

Burgwald and Giberson (15) applied the photelometer to both the Kay and Graham and Scharer's laboratory test, with good results, and in some instances it proved more effective than the naked eye. Its accuracy depends only upon the accuracy with which it is calibrated; once calibrated it will not change in its accuracy, while color standards may fade. Also light conditions may effect color comparisons, but not the photelometer.

APPLICATION OF THE PHOSPHATASE TESTS TO CREAM

All of the tests mentioned are applicable to the detection of irregularities in the pasteurization of cream as well as of milk (6, 7, 10, 13, 14, 16, 17, 18, 20, 24, 25, 26, 27, 28, 29).

In some instances slight modifications are mentioned. Kay and Neave (24) state "the most convenient method of applying the phosphatase test (to cream) is to dilute the cream with water until it contains some 4–6 per cent of butterfat, and then treat it as if it were milk."

The general procedure with all of the laboratory tests, however, is to apply the test to cream without dilution.

Scharer (13) states that in his rapid field test "if the butyl alcohol extraction is resorted to (for cream) it is usually sufficient to invert the test tube

4 or 5 times. Further inversion may extract fat adding yellow and green colors to the alcohol layer. Cream high in carotene may yield these colors."

It has been shown that the enzyme phosphatase is associated with the fat globules (6).

It has also been shown that cream separated either by gravity or mechanically from pasteurized milk gave higher and the skim lower phosphatase readings than the milk, and frequently such creams separated from properly pasteurized milks would give a reaction of being underpasteurized.

Raw cream was also reported to contain a higher phosphatase content than the milk from which it was separated (28).

This shows the necessity for proper mixing of sample to get the fat distributed before making test.

No difficulties were encountered with cream pasteurized after separating (25, 28).

Giberson (14) found that creams pasteurized in the laboratory at 143° F. for 30 minutes frequently gave phenol values indicating underpasteurization; but creams pasteurized at 145° F. for 30 minutes always gave tests indicating proper pasteurization. He used high butterfat creams, however, averaging 42.0 per cent butterfat.

PRACTICAL APPLICATION OF THE PHOSPHATASE TESTS TO PASTEURIZED MILK AND CREAM

The phosphatase test has been proven to be one of the most valuable tests ever given to the dairy industry. It ranks in value with the Babcock test; and the field test is just as simple to run.

The test has found wide application in the control of pasteurization of milk and cream both here and abroad, and the results have been rather amazing (7, 10, 14, 16, 17, 18, 19, 20, 23, 24, 29, 30, 31, 32, 33, 34).

Gilcreas and Davis (18) report that routine tests made on 780 samples of milk and cream labeled "pasteurized" collected from delivery wagons in various communities that 212 or 27.2 per cent were found to be underpasteurized, and of these 59 reacted as raw milk. Plant inspection following the reporting of results found the cause in all but 32 instances.

Tiedeman (30) reported that on more than 2,000 samples of pasteurized milk and cream examined 29 per cent were found to be unsatisfactory. Followup inspections resulted in plant operators admitting cause or inspector finding or securing evidence of cause in all but a few instances. In these cases a checkup on the process claimed to have been employed gave results indicating proper pasteurization.

Krueger (31) reported that more than 80 per cent of the positive tests found were on samples which had been processed on Sundays and holidays, indicating the cause to be more or less willful on the part of the operator of the equipment. A year later he reports (33) dishonesty on the part of some operators had been greatly reduced, and the number of samples of improperly pasteurized milk and cream found were very small. Investigation of these cases found the cause to be due primarily to the use of improperly constructed equipment, valves being the chief cause. In 88 plants inspected 212 outlet and 146 inlet valves were found defective, most generally due to grooves being too small to function properly.

Scharer (10) reports that during the first month the test was in operation 23,977 pounds of milk and cream were condemned as improperly pasteurized and excluded from the New York City market.

Pincus (32) reports that the New York City Health Department examined 10,635 routine samples of milk and cream from April 1 to September 20, 1937, and found 4.7 per cent showed evidence of being underpasteurized to a greater or less extent.

Rice (34) reported that during the past year (1938) in New York City no gross irregularities have been found and minor irregularities occur in about 2 per cent of the samples examined. Inspectors follow up all reports of irregularities. Most cases of irregularities have been found in cream. Cases are seldom due to willfulness, but usually to short comings of equipment or to factors of which the operator was not aware and which could not be easily detected by routine physical inspection of the plant.

Giberson (14) and Burgwald (29) report that of 429 samples of pasteurized milk from all over the State of Ohio tested during the past year (October, 1937 to August, 1938) at the Department of Dairy Technology, Ohio State University, 19.5 per cent were found to be underpasteurized. While 117 samples of the pasteurized milk from one city where the test was conducted as a routine measure and results reported, only 5 per cent of the samples were found to be underpasteurized.

Hoy and Neave (23) (England) report that a survey showed considerable improvement in the efficiency of pasteurization during the past two years.

The results above mentioned show that there was vital need of the phosphatase test and that the test is proving its reliability in the many checkups which have been made when irregularities have been shown by the test. These checkups when made have invariably resulted in locating the cause of the irregularities. Where the test is being used routinely the number of irregularities have dropped decidedly.

The causes of irregularities found both willful and otherwise are too numerous to mention, but are listed in the following references (10, 14, 16, 17, 18, 19, 23, 24, 30, 31, 32, 33).

In view of the fact that a reduction of 96 per cent in the phosphatase activity means all pathogenic organisms which may have been present in the milk prior to pasteurization have been destroyed, then a test showing slight underpasteurization is not serious.

APPLICATION OF THE PHOSPHATASE TEST TO HOMOGENIZED MILK AND TO CHOCOLATE DRINKS

Kay and Neave (24) report that the Kay and Graham phosphatase test may be applied to homogenized milk.

Hahn and Tracy (22) report using Scharer's laboratory test that homogenization by pressure had no effect on the phosphatase test.

The writer (unpublished) has observed that homogenization had no effect on either the Kay and Graham or Scharer's improved field test. It was noted, however, that at times the butyl alcohol layer showed a yellow color on the homogenized milk, when the milk was homogenized after pasteurizing, which was not obtained on the milk before homogenizing.

Scharer (10, 28) reports that his laboratory test can be applied to chocolate drinks without any change in procedure, or special treatment, other than to make a control determination when a positive result is obtained. This is done by repeating the test with the substitution of buffered water in the place of the buffered substrate. (No incubation necessary.) Any blue color developing would be due to interfering substances (added flavoring or color), then by difference, the amount of enzyme action can be evaluated.

Scharer (28) reports that his improved rapid field test may be substituted for the laboratory test. Interfering substances to be evaluated by using a control, substituting buffered water for the buffered substrate.

Using this control procedure any of the phosphatase tests may be applied to chocolate drinks.

APPLICATION OF THE PHOSPHATASE TEST TO OTHER DAIRY PRODUCTS

Butter

The various phosphatase tests have been applied to butter (6, 7, 13, 24, 28, 35, 36, 37). The application of the technic is the same as when applied to milk or cream with the exception of taking the sample. The most acceptable manner of preparing samples is to take a small quantity of butter to be tested (about 30 gr.) place in a centrifuge tube, melt at 40° C. and centrifuge. Make test on aqueous layer (serum) substituting it for milk in the test. Kay and Graham (7) and Kay and Neave (24) report that using this method a blue color greater than 2.3 Lovibond blue units would indicate raw or poorly pasteurized cream. They confirmed this with butter made from cream pasteurized in the laboratory at 145° F. for 30 minutes and with butter made in New Zealand from cream pasteurized by the flash method generally used there.

Scharer (13, 28) reports that butter made from cream pasteurized at 141° F. for 30 minutes or from correctly pasteurized cream to which raw cream had been added could be detected by his improved rapid field test. Using the laboratory method he reports (28) that butter made from "holder" pasteurized cream exhibited only slight phosphatase activity while butter made from "flash" pasteurized cream exhibited considerable phosphatase activity.

Storage conditions had little effect on the enzyme activity. This agrees with Parfitt and Brown (37). He also reports some brands of butter consistently show no phosphatase activity while others were surprisingly high. All were labeled "made from pasteurized cream."

Shadwick and Parker (35) report that butter freshly made from sour cream which had been adjusted to the proper acidity and flashed at 185° F. or higher gave negative phosphatase tests by both the Kay and Graham method (24 hour incubation) and by Scharer's modified field test. Lower temperatures were not satisfactory.

In Scharer's method they increased the incubation time from 10 minutes at 100° F. to 15 minutes at 105° F. which seemed to give closer agreement with the longer Kay and Graham test. Amyl alcohol was used with the Scharer test.

Over 90 per cent of the butter samples giving a negative phosphatase test by Scharer's method, when subjected to the keeping quality test, (holding 8 days at 70° F.) almost invariably gave a positive reaction at the end of that time. Indications were that the phenol production was due to bacterial action. Parfitt and Brown (37) report that their experience with bacteria would lead them to believe that the production of phenol by micro-organisms is rather uncommon. In any case, the use of a control test substituting buffered water in place of the buffered substrate and subtracting the phenol value from the incubated test, the amount of actual enzyme action could be determined.

Brown and Parfitt (36) report that samples of butter having a negative phosphatase test held up better under the keeping quality test of 10 days at 15.4° C. than did butter having a positive test.

Parfitt and Brown (37) believe that the phosphatase test for butter is of great value to the dairy technician to detect inadequate pasteurization of the cream, but doubts that the test should be used as yet by the public health control official in determining whether butter has been made from properly pasteurized cream. More experimental work is needed (35, 37).

Butter from plants using vat pasteurization showed less phosphatase activity than butter from plants using the flash system. In the flash system cream may go through too fast and not all be heated properly, as shown in one instance of a plant whose butter was frequently showing a positive phosphatase test. Upon investigation it was found that the cream was being heated to 190° F. but was going through so fast that not all was heated to that temperature. The result was that that cream not heated properly was the cause of positive tests.

Excessive washing of butter made from improperly pasteurized cream may cause it to give a negative test (37).

Cheese

Very little work has been reported on cheese. Kay and Neave (24) report experiments are in progress. So far they have found that there is a considerable quantity of phosphatase in cheese made from raw milk for at least two months after manufacture.

Scharer (13, 28) reports phosphatase activity present in cheese made from raw milk even after 18 months of storage, but was absent in cheese made from correctly pasteurized milk. Aging under storage conditions or prolonged ripening at room temperature has no effect on the phosphatase values. He found the best method to sample cheese for the phosphatase test, because of its high acidity, was to take equal portions of cheese, buffer, and water, mix by grinding, and then proceed as for milk. Using a control substituting buffered water for the buffered substrate will indicate the presence of phenolic contamination.

Ice Cream

Considerable work has been done on ice cream and ice cream mix with reference to the application of the phosphatase test (13, 28, 38, 39, 40, 41).

A higher pasteurizing temperature is necessary for ice cream mix than for milk or cream due to the apparent protective action of the additional solids (39, 40).

Caulfield and Martin (40) showed that the sugar in the mix was the protective agency against the inactivation of the enzyme; while Hahn and Tracy (39) showed that increasing the serum solid content had no effect on the inactivation of the enzyme.

Because of the sugar in the mix it was necessary to pasteurize at a temperature above 145° F. for 30 minutes in order to inactivate the enzyme. Hahn and Tracy (39) report that it is necessary to pasteurize at temperatures above 147° F. for 30 minutes. Caulfield and Martin state that a temperature of 150° F. for 30 minutes will give negative results regardless of the sugar content present.

A temperature and holding time sufficiently high and long enough to inactivate the enzyme should be employed, as ingredients that have a protective agency for the enzyme will undoubtedly also act as a protective agency toward destruction by heat of the bacteria present. At least 96 per cent of the enzyme must be inactivated to make certain that any pathogenic organisms which may have been present have been destroyed (6).

Nelson, *et al.* (41) showed that the enzyme phosphatase in ice cream mix is more resistant to heat than are the more heat resistant strains of *Escherichia-Aerobacter* organisms.

Certain flavoring and coloring materials added to ice cream may effect the test (13, 28, 38, 39, 40).

Pure vanilla extracts have no effect (38, 39, 40) except when vanilla bean (concentrate) was added in double the recommended amounts it had a slight effect (40).

The use of vanillin and coumarin crystals had a decided effect, coumarin more so than vanillin (38, 39). Other flavoring extracts, such as strawberry, cherry, maple, etc., had little or no effect, while the addition of "cold pak" fruits, such as cherry, strawberry, and pineapple increased the phenol value, and this value continued to increase slightly during storage (39).

Added colorings had such varied effect that no definite conclusion could be reached (39).

None of these substances should present any difficulty (13, 38) if a control test substituting buffered water in the place of the buffered substrate is made on all samples giving a positive reaction. The blue color developing in the control will indicate the extent of the indophenol color due to interfering substances, and by subtraction, the amount of enzyme activity may be obtained. Fruits and nuts if present should be strained out before making the test. Colored ice creams are best tested by a laboratory test rather than by the field test as coloring agent may be soluble in the alcohol used in extracting the indophenol. Fresh pack fruits and raw fruits and nuts may introduce both enzyme and phenol-like substances (38). This may be ascertained by heating a portion of the mix to a high temperature and compare the resultant test and control with those of an unheated portion.

Storage of ice cream made from properly pasteurized mix for two to twelve weeks had no effect on the phosphatase test (38, 39, 40). There was a slight reduction in phenol value at the end of 12 weeks in ice cream made from underpasteurized mix (38, 39).

SUMMARY

It has been shown that the enzyme phosphatase is present in all raw samples of milk, and that its thermal resistance is greater than that of any pathogenic organism which may be present in milk.

Due to its inactivation at pasteurizing temperatures generally employed, it adapts itself ideally to detecting irregularities in the pasteurization of milk.

The varous laboratory tests are of approximately equal value, and will detect variations as follows:

1.0° F. in temperature

5.0 minutes in holding time

0.1 per cent added raw milk to properly pasteurized.

The modified field test is almost as sensitive; and increasing the incubation time to 30 minutes will probably make it just as sensitive as any of the laboratory tests. It has the advantage of being able to be run directly in the plant in a very short time. Irregularities can be detected at once, shown to operator, and cause remedied.

A photelometer may be used with the laboratory tests for greater accuracy in some cases.

The test is very sensitive and precautions listed for each test must be followed closely.

The test may be applied to cream, and homogenized milk without modification.

For chocolate drinks and various ice creams it is necessary to run controls using buffered water in place of buffered substrate to detect the presence of any interfering substances.

For cheese and butter special preparation of the samples are necessary. More work needs to be done with the test on these two products.

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IMPROVEMENT IN THE VISCOSITY OF PASTEURIZED CREAM THROUGH SUBSEQUENT HEAT TREATMENT

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A commercial process of alternately warming and cooling pasteurized cream for the purpose of increasing its viscosity was first described by Hening and Dahlberg in 1932 (1). Since then, one French, (2) one German (3) and three United States Patents (3, 4, 5) have been granted on various features of the process. With the exception of the Nair and Mook patent (U. S. 5) each of the other patents is concerned principally with the production of an increase in the viscosity of cream after pasteurization. The Nair and Mook patent specifies optimum conditions for obtaining "maximum improvement in the desired characteristics" which include "viscosity, whipping quality, serum separation, elimination of plug, cream line and proper control of bacterial growth." The objective of all of these published studies, however, has been the control of processing conditions to bring about a desired specific effect in the milk or cream.

Although it had been noted in the investigative work leading to the U. S. patent by Nair and Mook that all creams did not respond alike to a specific warming and cooling treatment, no data were available to determine which of the constituents of the milk or cream was responsible for these variable effects. The study reported in this paper was undertaken for the purpose of ascertaining which of the cream constituents was involved in the process.

For simplicity's sake, the described process of alternately warming and cooling pasteurized cream to improve its viscosity and other properties has been referred to in the Borden Laboratories as the "rebodying" process. The term "rebody" therefore, will be used throughout this paper to designate that process.

In undertaking the study, three major questions presented themselves; 1. was the fat phase or the aqueous phase of cream more important in the rebodying process, 2. was the composition or source of the butterfat a factor in the process, and 3. which of the plasma constituents of cream were helpful or injurious to the process.

The simplest method of studying each factor appeared to be an investi-

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gation of the effect of the rebodying process on artificial creams made by dispersing melted butterfat in an aqueous phase containing various milk solids. In making the dispersions, butterfat from four localities in the United States was used. All of the constituents of the aqueous phase, however, were derived from milk or cream produced in central New York.

The data reported in this paper do not include all of the results with each artificial cream but are representative of three or more experiments with each kind of artificial cream.

EXPERIMENTAL PROCEDURE

The dispersing materials for the artificial creams were prepared as follows:

Sweet Rennet Whey. Sweet rennet whey was prepared in an experimental laboratory water-jacketed vat by adding 50 ml. of a 1:50 solution of rennet to two gallons of pasteurized skim milk warmed to 95° F. The curd set in about an hour, was cut with cheese knives and the whey heated to 130° F. The clear whey was siphoned off, cooled and half of it concentrated under vacuum. The concentrate was adjusted to 8.5–9.0 per cent solids before being used. The remaining half of the whey was dialyzed in cellophane bags against running tap water for 18 hours, concentrated under vacuum and diluted back to 9 per cent solids before being used.

Normal Sweet Cream Buttermilk. Normal sweet cream buttermilk was obtained by churning freshly pasteurized sweet cream. The buttermilk was passed through a DeLaval separator to remove unchurned fat and butter particles. It was used in normal concentration for making artificial creams. The total solids content was 8.5 per cent.

Washed Cream Buttermilk. In preparing washed cream and washed cream buttermilk, ten gallons of freshly separated heavy cream were diluted with twenty gallons of tap water at 105° F. in a thirty-gallon Cherry-Burrell spray vat, mixed, and separated in a DeLaval separator. Six gallons of cream were recovered. This was diluted again to thirty gallons with tap water at 105° F., mixed, and reseparated. The fat content of the cream washed in this way was 64–66 per cent. Although the above treatment was considerably less than the number of washings applied previously in studies on the isolation and composition of the material adsorbed on the surface of fat globules in cream, the object of the present experiment was not to wash away all of the materials present on the fat surfaces with the exception of those most closely adsorbed, but only to remove the major part of the plasma solids-not-fat in the cream. Rimpila and Palmer (6) have shown that the greatest decrease in phosphorus and proteins in cream occurs after the first washing.

The washed cream was pasteurized in the spray vat at 145° F. for thirty minutes, cooled to 50° F., churned, and the buttermilk and first butter washing recovered. These were passed through a DeLaval separator to remove fat and butter particles and the separated buttermilk concentrated in vacuum. The washed cream buttermilk used for making the artificial creams contained 2 per cent of solids.

Artificial Creams. In preparing the artificial creams, the butterfat was obtained by melting sweet cream butter and decanting the practically clear butter-oil. Sufficient melted butterfat to give a 35 per cent cream was added to the aqueous emulsifying material, the mixture warmed to 105° F., and passed through a small viscolizer at 200 pounds three successive times. The viscolizer used was a No. 0 Union Steam pump machine equipped with a Duo-Visco valve having a No. 3 breaker ring. When not too heavy, the cream was cooled over a small surface cooler; otherwise it was cooled by setting the container in running tap water.

A microscopic examination was made of each cream to insure that it was very nearly like normal cream in degree of fat dispersion before the rebodying process was applied.

Three or more of each of the following emulsions were prepared:

Minnesota butterfat in New York normal fluid skim milk.
Minnesota butterfat in New York reliquefied skim powder.
Minnesota butterfat in New York condensed sweet rennet whey.
Minnesota butterfat in New York condensed dialyzed rennet whey.
Minnesota butterfat in New York normal sweet cream buttermilk.
Minnesota butterfat in New York condensed washed cream buttermilk.
New York butterfat in New York condensed washed cream buttermilk.
New York butterfat in New York normal sweet cream buttermilk.
New York butterfat in New York condensed dialyzed rennet whey.
Michigan butterfat in New York condensed washed cream buttermilk.
Texas butterfat in New York washed cream buttermilk.

It was possible to prepare a uniform 35 per cent cream in every case. However, some of the creams were thicker than others and not all of them were stabile after pasteurization, *i.e.,* the emulsion either broke during pasteurization or the cream took on a curdled appearance. In no case was the acidity of the cream greater than 0.13 per cent.

All of the creams were pasteurized and rebodied in a small laboratory experimental vat under the same experimental conditions as to rate and temperature of heating and cooling and degree of agitation. Two samples could be treated at the same time under identical conditions. Pasteurization was carried out by holding for 30 minutes, followed by surface cooling to 45° F. The rebodying process consisted of immediately warming the pasteurized samples to 86° F. in 20–25 minutes, vat cooling from 86° F. to 65° F. in 12–15 minutes, followed by surface cooling to 45° F. After 24 hours storage at 40° F., the viscosity of the creams was measured in seconds flow time at 60° F. with the Borden Body Flow Meter (7).

Characteristics of the creams and typical effects of the rebodying process are given in Table 1.

TABLE 1

Effect of different plasma ingredients on the rebodying process of artificial creams

Source of butterfat	Stabilizing material from New York milk and cream	Acidity of artificial cream	Viscosity flowtime		Increase in viscosity
			After pastzn.	After rebodying	
		Per cent	*Seconds*	*Seconds*	*Per cent*
Minnesota	Normal fluid skim	0.12	206.0	201.2	None
"	Reliquefied skim powder (Low percentage of soluble lactalbumin)	0.13	710.0	679.0	None
"	Condensed sweet rennet whey (Lacking casein, but high in lactalbumin, lactose and milk salts)	0.11	62.0	57.5	None
"	Condensed dialyzed rennet whey (High percentage of lactalbumin, low percentage of lactose and milk salts and lacking casein)	0.11	87.7	76.7	None
"	Normal sweet cream buttermilk (Fat membrane material plus constituents of normal fluid skim)	0.11	curdled during pasteurization		
"	Washed cream buttermilk (contains only fat membrane material)	0.09	85.6	319.8	273
New York	Washed cream buttermilk	0.05	70.0	186.8	167
" "	Normal sweet cream buttermilk	0.12	curdled during pasteurization		
" "	Condensed dialyzed rennet whey	0.10	111.5	Badly churned	
Michigan	Washed cream buttermilk	0.03	56.5	95.1	68
Texas	" " "	0.03	61.7	119.1	93

A study of the results of the rebodying process on the artificial creams reported in Table 1 shows definitely that none of the constituents of normal fluid skim milk are capable of stabilizing butterfat in such a way as to make a cream which would respond to the rebodying process. Increase in viscosity of the artificial creams resulted only when the butterfat was stabilized with the membrane material of normal milk fat globules. This was true regardless of the source of the butterfat. However, it was found that the emulsions prepared with Michigan and Texas butterfat in washed cream buttermilk were much less stable than those prepared with New York or Minnesota butterfat. In fact, two of the three emulsions prepared in the former cases churned so badly during the rebodying process that viscosity measurements could not be made. In the creams prepared with Michigan and Texas butterfat which are reported in Table 1 the percentage increase in viscosity after rebodying is also less than for the Minnesota and New York butterfat creams. This would indicate that the composition of the butterfat is a factor in the rebodying process. At least, it is a factor in the stabilization of the emulsions.

Failure to prepare 35 per cent butterfat emulsions in normal sweet cream buttermilk which were stable enough to undergo the rebodying process, may have been due to the low percentage of the fat globule membrane material. The normal buttermilk used in normal concentration contained a relatively small percentage of "membrane" material, whereas the washed cream buttermilk, containing 2 per cent of solids, was composed entirely of fat "membrane" material. When the above experiment with normal sweet cream buttermilk was repeated using 20 per cent of butterfat, more stable emulsions resulted and the creams had a slight response to rebodying (an increase of 18 seconds in the rebodied cream).

Addition of Skim Milk Solids to Washed Cream

Results of the effect of rebodying normal 35 per cent central New York cream before and after the removal of most of the solids-not-fat of the cream, showed that washed cream still responded well to the rebodying process. In order to check again the specific effect of the solids-not-fat in normal cream, skim milk solids and concentrated dialyzed rennet whey were added to washed cream before pasteurization.

Freshly separated heavy raw cream was washed twice in a spray vat as previously described. When the washed cream, containing 66 per cent butterfat, was reduced to 35 per cent butterfat with either reliquefied skim milk powder or condensed dialyzed rennet whey, then pasteurized and rebodied, the viscosity of the rebodied cream was greater than that of the washed cream itself. The viscosities of the original cream, the washed cream, and the washed cream with added solids after pasteurization and after rebodying are shown in Table 2.

TABLE 2

Effect on rebodying of adding plasma or serum solids to washed cream

	Viscosity flowtime		Increase in viscosity
	After pasteurization	After rebodying	
	seconds	*seconds*	*per cent*
Normal cream	114.4	375.5	228
Washed cream	56.7	159.2	180
Washed cream and reliquefied skim milk powder	80.3	252.9	214
Washed cream and dialyzed whey	66.4	257.5	287

Although no response to the rebodying process was brought about when one or more of the constituents of the aqueous phase of milk was used to stabilize the butterfat in the artificial creams, the presence of these constituents in the aqueous phase of normal cream appears to play a part in the amount a cream responds to the rebodying process. This was evidenced by a lower percentage increase in the viscosity of rebodied washed cream as compared with unwashed cream and also by the greater percentage increase in the viscosity of rebodied washed cream to which skim milk solids had been added. Lactalbumin had a very definite effect.

Addition of Fat Membrane Material to Artificial Creams

Since the presence of the fat membrane material of normal cream played such an important part in the rebodying of artificial creams, as well as in washed cream, when used as the fat stabilizing material, concentrated solutions of washed cream buttermilk were added to other artificial creams in order to see whether beneficial effects could also be produced when the membrane material was present in the aqueous phase of the cream and not as the fat stabilizer. For this purpose, both New York and Michigan butterfat were dispersed in dialyzed sweet rennet whey to produce 40 per cent creams. These were standardized to 30 per cent butterfat with normal skim milk and concentrated washed cream buttermilk.

TABLE 3

Effect on rebodying of adding washed cream buttermilk solids to artificial cream stabilized with serum solids

	Viscosity flowtime	
	After pasteurization	After rebodying
	seconds	*seconds*
New York butterfat stabilized with serum solids, washed cream buttermilk added	44.6	48.5
Michigan butterfat stabilized with serum solids, washed cream buttermilk added	45.2	48.5

The artificial cream made with New York butterfat and sweet rennet whey, and standardized with skim milk, churned during the rebodying process. When Michigan butterfat was substituted for New York butterfat, the pasteurized artificial cream churned too badly to be subjected to rebodying. In the case of the two creams standardized with washed cream buttermilk, the addition of the fat membrane material in the aqueous phase of the cream, prevented the creams from churning during pasteurization but had practically no effect on the viscosity of the rebodied creams. The increases in viscosity after processing were negligible, as shown in Table 3.

SUMMARY AND CONCLUSIONS

1. The fat phase of normal cream plays the most important rôle in the cream rebodying process.

2. The fat membrane material of normal milk fat globules was found to be the most effective butterfat stabilizer for artificial creams and always resulted in a cream which would respond to the rebodying process.

3. Stabilization of butterfat with part or all of the constituents of normal skim milk did not yield artificial creams which would respond to rebodying, although the rebodying process as applied to other fat emulsions than cream has been patented (4). None of the emulsions prepared with other than the normal fat globule membrane material in these experiments responded to the process.

4. The constituents of the aqueous phase of normal cream exert a beneficial effect on the rebodying process but this effect is not noted except when the butterfat is stabilized by its normal membrane.

5. The composition of the butterfat appears to be a factor in the stability of cream subjected to the rebodying process.

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THE FLAVOR OF MILK AS AFFECTED BY SEASON, AGE, AND THE LEVEL OF FEEDING DAIRY COWS

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An investigation with the station Jersey herd on the relation of input to output in milk production, in cooperation with the United States Department of Agriculture, afforded an excellent opportunity to study the effect of different levels of feeding upon milk flavors.

The object of the cooperative experiment was to determine the rate and method of feeding which is most profitable under different price conditions. The cows selected were divided into six comparable groups. The control group was fed at the Morrison Standard level. The other groups were fed at levels of 20 and 10 per cents below standard and 10, 20 and 30 per cents above standard. The roughage consisted of alfalfa hay (15 pounds daily per 1000 pounds body weight), corn silage (20 pounds daily), and mangels (15 pounds daily) and in the summer green cut alfalfa and green cut soy beans. The feeding of green cut legumes was started May 24th and discontinued September 27th. A good grain mixture of about 18.5 per cent crude protein was fed.

At the beginning of the experiment there were 6 cows on the – 20 level, 3 cows on – 10, + 10 and + 20 levels, and 6 cows on the + 30 level. Before the first year of the experiment was completed some of the cows were sold from the herd due to causes not resulting from the experiment.

The cows were machine milked three times a day and fed grain three times a day. Hay was fed twice a day and mangels and silage once a day. The feeds, of course, were all analyzed and the amounts fed were weighed and any feed not eaten was weighed back. The high producing Jerseys of the station herd could not always consume enough food to meet the requirements of the higher levels of feeding and in two cases were changed to lower levels. These changes were taken into consideration in comparing the flavor scores of the milk. However, some of the cows at the higher levels of feeding included in this experiment, even though they were fed to the limit of their capacity, were not meeting the requirements of the level of feeding.

The experimental cows in the spring, summer and fall were on an exercise plot which furnished no appreciable pasturage since it was necessary to weigh and analyze the green feed they received.

The judging of the flavor of the milk from the individual cows was continued for one year. The milk was judged at the start of the experiment at weekly intervals, then every two weeks and once a month for the last few

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months of the experiment. The changes in flavor were not rapid enough to indicate the necessity of scoring the milk from individual cows every week. The flavor of the milk from all of the cows in the Jersey herd was scored whether or not they were on the experiment.

The study of the flavors for a complete year also offered an opportunity to study the effect of seasonal variations on the development of oxidized flavor. Likewise, since six heifers freshened during the year the flavor of their milk could be compared with that of the rest of the herd.

The experimental procedure was similar to that previously used (1). Samples of approximately one quart of milk from each cow were taken at the noon milking. One pint bottle of each of these samples was pasteurized at a temperature of 143° F. to 145° F. for one-half hour in a water bath. After pasteurization half of each pint of hot milk was poured into half pint bottles containing sufficient copper sulphate solution to increase the copper content by 0.25 p.p.m. It was considered to be desirable to add copper to part of the milk to determine more accurately the degree of susceptibility of the milk from each cow to the production of oxidized flavors. The milk was all cooled in the bottles in an ice-water bath and stored at 35 to 40° F. The raw milk was judged for flavor by 2 or 3 members of the department* shortly after milking and the raw, pasteurized and pasteurized plus copper milks were judged the first, third, and fifth days after milking and pasteurization. The flavor scores made on the fifth day are not included since the results are similar to those of the third day.

RESULTS

The Effect of Various Feeding Levels

Table 1 gives the average scores of the raw milk from individual cows judged the first and third day after milking and the pasteurized milk and pasteurized milk plus 0.25 p.p.m. copper judged the first and third day after milking. The average flavor scores given in this table show that the different levels of feeding have no influence on the flavor of the milk. The average flavor scores of milk from cows on the higher levels of feeding appear in some cases to be a little higher but this difference is not great enough to be considered significant, since other factors would account for some difference. The flavor of the milk from some groups of cows picked in this way would tend to be somewhat better than the flavor of the milk from other groups. The average flavor scores of the raw milk the first day after milking are practically the same for the -20 and $+30$ levels and the flavor scores for the pasteurized milk the first day after pasteurization are very much the same for each feeding level.

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* The milk was scored for flavor by the authors and J. C. Marquardt working independently but some samples were not scored by the Junior author. The fresh raw samples were often judged by the Senior author alone.

TABLE 1

Average flavor scores for milk from cows of Jersey herd on various feeding levels from January 1937 through December 1937

Feeding level	No. of cows	Average flavor score of milk					
		Raw		Pasteurized		Past. plus 0.25 p.p.m. copper	
		1st day	3rd day	1st day	3rd day	1st day	3rd day
– 20	6	22.2	20.9	21.6	19.8	20.4	16.5
– 10	2	20.4	20.5	22.2	19.6	20.1	16.0
Standard	3	22.0	20.7	21.6	19.0	20.0	16.2
+ 10	3	22.4	20.6	21.7	19.6	18.5	15.4
+ 20	3	22.8	21.9	22.4	21.1	20.7	16.6
+ 30	4	22.5	21.4	22.2	20.4	20.8	15.9

Table 2 gives the percentage occurrence of oxidized flavor in the pasteurized milk and pasteurized milk plus 0.25 p.p.m. copper for the first and third days after pasteurization for the cows on the various feeding levels. Here again there is no correlation between the percentage occurrence of oxidized flavor and the feeding level. As can be seen from table 2 in the pasteurized milk the first day after pasteurization no oxidized flavors occurred in the – 10 and + 20 groups and the percentage occurrence in the – 20 and + 30 groups was practically the same.

TABLE 2

Percentage of samples of milk from cows of Jersey herd on various feeding levels that became oxidized from January 1937 to December 1937

Feeding level	No. of samples	Pasteurized		Past. plus 0.25 p.p.m. copper	
		1st day	3rd day	1st day	3rd day
– 20	62	1.6	21.1	29.0	78.8
– 10	20	0.0	31.5	45.0	84.2
Standard	46	2.0	26.0	47.8	73.9
+ 10	34	17.0	42.4	55.8	90.9
+ 20	47	0.0	8.5	36.1	89.5
+ 30	51	1.9	18.0	24.4	83.6

Occurrence of Oxidized Flavors Due to Seasonal Variations

Considerable experimental evidence has shown that during the winter months the development of oxidized flavors greatly increases in milk and after the cows are on pasture or are fed green feed this flavor practically disappears. There is generally a carry over in the effects of the pasture or green feed for 15 days to a month after it has been discontinued.

Kende (2), Guthrie and Brueckner (3), Thurston (4), Greenbank (5), Anderson (6) and Dahle (7), have shown that there is much less tendency for the development of oxidized flavors in milk produced in the summer months than that produced in the winter months. However, Kende states that "There is no definite correlation between the presence of the reducible material in milk and the feed supplied to the cows."

The seasonal trend in the development of oxidized flavors was followed in this experiment in the raw and pasteurized and pasteurized plus copper milks. Figure 1 gives the percentage occurrence of oxidized flavors in the milk from all of the cows of the station Jersey herd from January, 1937, through December, 1937. The percentage occurrence of oxidized flavor is shown in graph form for the milk from individual cows the first and third day after pasteurization without added copper and with 0.25 p.p.m. copper added. The raw milk first day after milking is not shown since only one sample developed an oxidized flavor the first day after milking for one scoring period only.

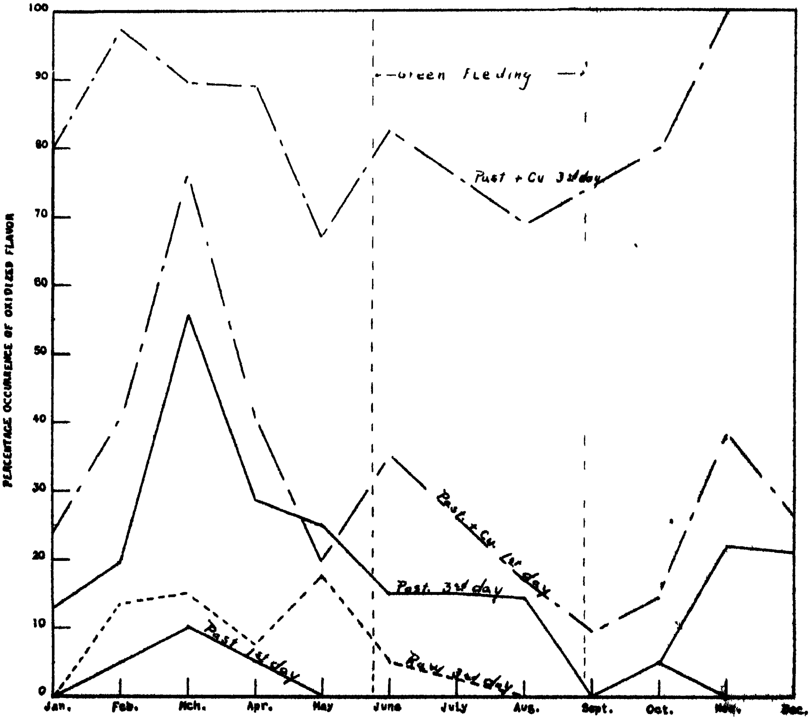

FIG. 1. Percentage occurrence of oxidized flavors in milk from station Jersey herd from January to December 1937.

The occurrence of oxidized flavor for the pasteurized milk the first day after pasteurization, as shown in figure 1 reached a high in March and was absent during May, June, July, August and September and recurred in October. The percentage oxidation curve for the raw milk judged the 3rd day after milking was more variable than for the pasteurized milk judged the first day after pasteurization. The percentage occurrence of oxidized flavor started down in April, went up again in May, was zero in August and September and increased again in October and November. The raw and pasteurized milk judged in the flavor experiments the preceding fall did not show this increase in oxidized flavor in October and November. The milk scored on the third day after pasteurization shows somewhat the same general trends as that scored the first day after pasteurization but of course the percentage occurrence of oxidized flavor is very much greater. The pasteurized milk plus 0.25 p.p.m. copper on the first day shows a considerably higher percentage occurrence of oxidized flavor than the pasteurized milk without copper on the third day. The pasteurized milk plus copper on the third day ranges from 55 per cent oxidized flavor in September to 100 per cent in November and December. Both of the pasteurized plus copper curves go down in May and then for some unknown reason go up in June and then drop off to a low in September.

Since the period in which the cows received green feed was from May 24 to September 27 the change in the percentage occurrence of the oxidized flavor cannot be attributed entirely to the change of feed. The percentage occurrence of oxidation started to drop off in April prior to the time the cows received any green feed.

Occurrence of Oxidized Flavor in Milk of Heifers

During this experiment 6 heifers freshened and they were included in the experiment. Two of these heifers freshened the last of February, two in April, one the last of May and one the last of August. The milk from these heifers especially at the beginning of their lactation period seemed to be more susceptible to the development of oxidized flavors than the milk from other cows with the exception of the one freshening the last of August. Since this difference was noticeable at the time of scoring the milk, the percentage occurrence of oxidized flavor of the milk from these heifers has been compared with that of the milk from six mature cows picked at random for a similar seasonal period. Of 58 samples of pasteurized milk from the heifers scored the first day after pasteurization 4, or 6.8 per cent, were oxidized whereas none of the 57 samples from the cows were oxidized. The same comparison of the same milk the third day after pasteurization showed 14 out of 57 samples, or 24.5 per cent, oxidized for the heifers and 7 out of 58, or 12.0 per cent, oxidized for the cows. A comparison of this same milk plus 0.25 p.p.m. copper scored the third day after pasteurization showed that 84.4 per cent of the milk from the heifers was oxidized and 79.3 per cent from the cows. There are not enough results to prove that the milk from heifers is more apt to develop oxidized flavors than the milk from older cows but nevertheless the comparative difference is striking enough to be of considerable interest.

CONCLUSIONS

The flavor of fresh milk and its keeping qualities as indicated by the percentage occurrence of oxidized flavor and the numerical score in aged milk was not influenced by the level of feeding under the conditions of the experiment.

The occurrence of oxidized flavor was considerably less in summer months than in winter months but there was not a definite correlation between the exact period of feeding green cut legumes and the occurrence of oxidized flavors.

The milk from first calf heifers showed a higher incidence of oxidized flavors than milk from older cows but the data were not considered sufficient to draw definite conclusions even for the one herd in which all of the data for this study were obtained.

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ROUGHAGE QUALITY AND QUANTITY IN THE DAIRY RATION, A REVIEW

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INTRODUCTION

Roughages are characterized chemically by a relatively large percentage of crude fiber which forms the framework of the plant (13). According to Morrison (350) roughages are feeds which are high in fiber and therefore low in total digestible nutrients. Such feeds as hay, corn fodder, straw, silage and pasture belong to this class. Some of the low-grade milling by-products, such as oat hulls, ground corn cobs and cottonseed hulls are roughages rather than concentrates, because they are high in fiber and furnish very little nutrient per unit of dry matter. Roughages may also be defined as feeding stuffs which contain a relatively small amount of net energy (or digestible nutrients) in a large bulk and contain a large percentage of crude fiber. Roughages include the vegetative part of plants as well as feeding stuffs of low total digestible nutrient content on a dry basis.

Armsby (13) and Morrison (350) used the following classification of feeding stuffs: (a) roughages or coarse fodders; (b) roots and tubers, and (c) concentrates.

The dairy cow, because of her spacious digestive tract which is adapted to the handling of large amounts of roughages, is the most important domestic animal to consider in a greater roughage program. The trend toward soil conservation and the closing of the spread between the price of dairy products and feeding concentrates has stimulated a world-wide interest in greater use of home-grown roughages.

The importance of using more home-grown feeds, especially legume hay and corn silage, in the ration of dairy cattle was emphasized by Lane (290) in 1902. Fraser (155) stressed more milk per acre by using home-grown feeds, especially alfalfa and corn silage.

The magnitude of the roughage problem is shown by data reported in the U. S. Census of Agriculture for 1935, which is presented in Table 1.

The use of roughage as a feed for dairy cattle depends on the amount of roughage consumed, chemical composition, digestibility, balance of nutrients and economy. The literature pertaining to the subject matter of this review is so voluminous that a detailed survey cannot be given here.

ROUGHAGE CONSUMPTION

In order to utilize more roughage in the feeding of dairy cattle, further knowledge concerning the factors affecting the consumption of various

TABLE 1

Roughages grown in the United States

Total acres in farms January 1, 1935		1,054,515,111	
Pastures	1934	*Acres*	
Plowable pasture		97,579,038	
Woodland pasture		108,095,711	
Other pasture		311,225,652	
Total		517,890,401	(40% of total farm acreage)
Hay	1934		*Tons*
Alfalfa		11,669,135	18,742,098
Sweet clover and Lespedeza hays		2,564,667	2,306,944
Timothy and clover		19,978,691	16,346,092
Annual legumes saved for hay		9,500,946	7,970,423
Small grains cut for hay		6,980,258	4,920,725
All other tame and wild grass cut for hay		17,930,813	11,798,065
Total		68,624,510	62,084,347
Sorghums for forage			
Silage, hay and fodder		7,909,269	7,452,897
Corn for silage (1933)		4,425,000	

roughages is imperative. The food capacity or appetite of an animal is the total amount of dry matter actually consumed when the animal is offered as much as it cares to eat (354).

Food consumption is affected by factors inherent in the animal such as size, age, health, psychological factors, inheritance, exercise and milk production; as well as factors inherent in the feed and environment such as palatability, amount of food offered, bulk of the ration, balance of the ration, and environmental temperature.

Palatability of Pasture.—A vast amount of research has been devoted to the study of the palatability of different pasture plants, usually by the cafeteria method. Palatability has often been confused with the consumption of large quantities of dry matter. As has been pointed out, palatability is but one factor responsible for a large food intake. A food may be consumed in large quantities yet it may not be very palatable. For instance, a silage mixture of 20 parts sweet clover and one part corn was not relished by cows, although when forced to eat it, one cow consumed up to 94 pounds of this silage per day which contained 40 per cent dry matter (95). Two other cows consumed 60 pounds per day for 126 days and the fourth animal refused the silage entirely after 30 days. On the other hand, a food may be very palatable but a small amount may give the animal a feeling of satiety. Nitrogen fertilizers have been shown to increase the palatability of the grass (106) and lime has been shown to increase the palatability of pasture herbage (426, 67, 89). Godden (164) studied the chemical composition of the natural uncultivated pasture herbage actually "eaten" and herbage from the same area which was not "eaten." The grass which was eaten was higher in calcium, phosphorus, sodium, potassium, chlorine, and nitrogen and lower in fiber than the grass which was not eaten.

Winter grass compared with spring or summer grass was found to be unpalatable (553). Frost with its accompanying browning effect was the most important factor leading to the reduction in palatability.

Palatability is conditioned by mechanical state and not by taste (264). Reed canary grass which was the least palatable was found to be the least tough as measured by the breaking strength of the leaves (7). Semple and associates (430) state that palatability depends on the kind of plants, their tenderness, stage of maturity, density of the plants, and climatic and soil conditions. Livestock usually select plants which contain relatively high percentages of minerals.

Palatability of Hay and Silage.—Foster and Merrill (143) in 1889 and Mills (338) in 1896 reported that the relative palatability decreased as the alfalfa plant approached the fuller stages of bloom. Waters (501) using the free choice method with dairy cows reported that there were no significant differences in the consumption of the first three cuttings but that the fourth and fifth cuttings were less palatable. Cattle preferred white Australian wheat hay cut in blossom to hay of the same variety cut at later stages of development and such hay became less palatable as complete maturity was approached (219). Milking cows refused more of the second cutting than the first or third, and at times consumed the second cutting of alfalfa very reluctantly (66). Sotola (443) did not observe any difference in the palatability of the various cuttings of alfalfa. Prairie hay cut in July was more palatable to steers than that cut in October (71). The palatability of Sudan grass hay decreased with the increased maturity at the time of cutting when fed to dairy cattle (96).

Unfavorable curing conditions also decreased the palatability of Wyoming hay (521). Wyoming native hays cut early in the haying season or those which had plenty of water late in the season were as palatable as the best alfalfa. The palatability of irrigated alfalfa decreased with advance in bloom, with the exception of the second cutting. The relative palatability of Wyoming native hays was closely associated with the sugar content but alfalfa hay did not show this relationship.

The relative palatability of different U. S. grades of alfalfa was tested with steers. The palatability of the alfalfa hays used was independent of the grade of hay. The U. S. No. 3 hay which was coarse and stemmy was more palatable than the No. 1 and No. 2 hays (12).

The palatability of various silages was studied by Westover (515).

Palatability as an index of nutritive value of roughages has probably been overemphasized since it is not always a criterion of dry matter consumption.

Dry Matter Consumption on Pasture.—Although it is very important to know the amount of dry matter that cattle will graze under various conditions, there is a paucity of data in this regard. Helms (218) reported that two lots of six cows each were tethered on grass within a large measured circle. At the end of the day the grass left within the circle was cut and weighed. The trial lasted 14 days. The cows consumed about 155 pounds of grass per head per day. Milk production did not influence the amount of grass consumed since the dry cows ate as much as the heavy milkers.

Graves and associates (172) fed grass clipped from irrigated pastures to four Holstein-Friesian cows for periods of 15 to 55 days. The average daily consumption by individual animals for an entire period varied from 122 pounds for the smallest cow to 152 pounds for the largest cow. The results obtained with a cow which was switched back and forth from pasture to grass clippings indicated that cows do not consume as much dry matter while grazing as when fed grass clippings.

A cow will graze about six per cent of the immature grass stand on one acre of ground per day and the limit of her capacity is about 150 pounds of green grass or 30 to 35 pounds of dry matter depending on her size (555). Cows will eat enough immature grass when the pasture is at its best for maintenance and the production of well over a pound of butterfat daily.

Hay and Silage Consumption.—Dairy cows consumed more of the better grades of alfalfa, timothy and soybean hay than they did of the poorer grades (559). When cows were changed from immature grass to mature hay or silage, a prompt decline resulted in consumption and production (171, 172). More dry matter was consumed in the form of hay than as silage.

When different amounts of corn silage and silage of different dry matter content were fed, the cows adjusted their hay consumption to the quantity of dry matter consumed in silage. The total roughage dry matter intake was not greatly affected either by the silage quantity, the proportion of ear, total dry matter, or water in the ration (516).

Willard (521) found that cows increased their hay consumption with increased allowance and that the consumption of hay tended to increase with the increase in concentrate allowance and vice versa. He worked out a method of determining the index of a cow's intake of hay. Unfavorable curing conditions produced a decrease in consumption of Wyoming hays. The sugar content did not appear to bear a close relationship to the amount of alfalfa hay eaten while the opposite was true of native hays. There was no relationship between the grade and the amount of alfalfa hay consumed.

The average daily amounts of hay consumed by beef cattle in three winters as calves, yearlings, 2-year-olds were: alfalfa and cane hay 24.6 pounds; alfalfa and prairie hay, 22.4 pounds; alfalfa hay, 21.5 pounds; cane hay, 20.4 pounds; prairie hay and cane hay, 14.4 pounds; and prairie hay, 16.8 pounds. Beef calves fed alfalfa alone consumed 16.3 pounds while those fed native hay consumed only 10.2 pounds daily (250).

Reed and associates (401) placed heifers on alfalfa alone at six months of age. During the first and second years the heifers consumed an average of 7.8 and 15.7 pounds of hay daily respectively. A lot receiving alfalfa and corn silage consumed about the same amount of hay-equivalent as the lot on alfalfa alone. Both groups were subnormal in weight at two years of age. Willard (523) concluded that calves may make desirable growth when fed high quality alfalfa alone as early as nine months of age. Bender and Bartlett (41) fed 18 heifers from six months of age to first calving on roughage alone (hay and pasture). These animals weighed 1052 pounds before freshening and were normal in height. The Montana Station (345) reported that heifer calves after being weaned in the fall and given a full feed of alfalfa hay during the winter consumed 12.7 pounds per day. The next winter as 2-year-olds these heifers consumed 24.5 pounds of alfalfa per day.

Milking cows which ate 13 to 14 pounds of alfalfa daily consumed only 5.7 pounds of reed canary grass hay (16). The poor appetite for reed canary grass was attributed to lack of palatability. Knott and Hodgson (278) in experiments with 41 cows on an all roughage ration found that there was little correlation between the dry matter consumption and age or weight or between age or weight and production, but that there was a correlation between milk production and dry matter consumption. The cows used in this experiment consumed as dry matter 22.2 pounds of grass hay, clover hay and silage; 25.7 pounds of alfalfa hay; 19.2 pounds of grass and clover hay; and 18.3 pounds of silage per 1,000 pounds of body weight daily.

A phosphorus deficiency is manifested by a decreased appetite for roughage (248). Kleiber (275) emphasized the importance of determining food capacity on a standard ration to be used as a criterion for estimating the breeding value of an animal. He stated that it is reasonable to assume that systematic breeding on the basis of relative food capacity would result in an increase in the economy of animal production.

As the factors affecting dry matter consumption become known and the quality of hay and silage improve, the amount of roughage consumed may be expected to increase. The old thumb rules of feeding were based on the consumption of two pounds of roughage equivalent per 100 pounds of body weight. Consequently, a revision of the rules for roughage feeding means a revision downward of the old thumb rules for grain feeding and greater use of home-grown roughage. The results summarized in Table 2 suggest that most cows will consume from 2.5 to 3.0 pounds of hay per 100 pounds of body weight.

CHEMICAL COMPOSITION AND FEEDING VALUE OF ROUGHAGE

The chemical composition of roughages depends on the botanical composition, the stage of maturity, the type and fertility of the soil, the method of conservation, as well as the climatic conditions.

Effect of Stage of Maturity.—Deetz (97) in 1873 studied the chemical

TABLE 2

Roughage consumption by lactating dairy cows

Source of data	Kind of ration	Wt. of animal	Av. daily consumption				Av. daily roughage consumption per 100 lbs. body weight
			Hay	Silage	Hay equiv.*	Grain	
		lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
Kansas (401)	alfalfa alone 1st lact.	859	29.4				3.4
	alfalfa alone 2nd lact.	976	31.3				3.2
	alfalfa & corn silage 1st lact.	935	13.4	31.5	23.9		2.6
	2nd lact.	1077	15.4	39.2	28.5		2.6
	alfalfa, corn silage, light grain						
	1st lact.	1055	13.4	29.1	23.1	4.1	2.2
	2nd lact.	1112	12.8	32.3	23.6	5.3	2.1
	alfalfa, corn silage. heavy grain						
	1st lact.	983	11.7	23.3	19.5	5.2	2.0
	2nd lact.	1101	14.0	35.0	25.7	8.4	2.3
Nevada (212)	alfalfa alone (4 cows)	1355	36.9				2.7
	alfalfa and grain (4 cows)	1371	34.0			6.0	2.5
Oregon (258)	alfalfa alone (long) 14 cows	985	30.0			0.6	3.0
	alfalfa alone (chopped) 9 cows	1045	32.2			0.2	3.1
	alfalfa (long and grain) 7 cows	888	18.6			10.1	2.1
U.S.D.A. (173)	alfalfa alone (15 cows)	1226–1332	35.6–44.2				2.8–3.6
	full feed (15 cows)			24.4		11.4	
Ohio (343)**	heavy hay (Holsteins)		30.0	15.0	35.0	5.0	
	light hay (Holsteins)		15.0	42.0	29.0	8.4	
U.S.D.A. (172)	grass clipped, fed green 1929	1368			35.7		2.6
	grass hay	1369	40.4				3.0
	grass silage	1280			28.7		2.2
U.S.D.A. (171)	grass hay	1444	44.5				3.1
	grass silage	1335			33.4		2.5

* Hay equiv. = hay plus ⅓ of silage.

** alfalfa – timothy.

composition of perennial rye grass cut at various stages of development. The protein declined sharply from May 6 to May 20 and more slowly thereafter. The calcium and phosphorus also fell somewhat during this period but did not change much later.

Kellner (266) in 1879, Jordan (261) in 1882, and Wilson (525) in 1884 reported that the nitrogen and ash of the timothy plant were higher in the early stages of maturity and decreased in the ripening stages but that the reverse condition prevailed with respect to the nitrogen-free extract. Ladd (288) studied the chemical composition of the timothy plant at four stages of growth. He reported an increase in sugar and starch after blossoming. The important fact that immature grasses are high in protein and low in fiber and that the percentage of protein declines and the crude fiber increases with maturity was observed by Richardson in 1883 (406).

Morse (351) in 1888 cut timothy grass at various stages of maturity. The ash was more abundant during the rapid growth of the plant than after the growth had ceased. The ether extract decreased until blossoming, then increased until the seed began to form, when it again decreased, reaching its lowest point as the seed began to harden. The crude fiber increased steadily, with two exceptions, until the formation of seed, after which there was a slight decrease. The nitrogen-free extract remained nearly constant after the grass had nearly reached its full height. Crude protein steadily decreased with the development of the plants although it remained nearly constant after the bloom began to fade. Dry matter steadily increased until the plant formed seed. The young grass was richer in fat and protein, whereas mature grass was richer in carbohydrates or fiber and nitogen-free extract. Timothy yielded the largest amount of digestible protein when cut at the beginning of bloom. The total amount of digestible matter was largest when cut at the beginning of bloom. Armsby and associates (15) in 1888, Crozier (85) in 1894, and Ellett and Carrier (119) in 1915 determined the chemical composition of pasture grass cut at the grazing stage of growth. The protein varied from 12.4 to 21.9 per cent on the dry basis and the crude fiber varied from 18.2 to 41.2 per cent.

Mortimer and Ahlgren (352) investigated the chemical composition of pasture grass cut about every two weeks throughout the season, with and without various fertilizers. The grass usually contained about 20 per cent protein on the dry basis.

The composition of blue grass cut at different stages of maturity was studied by Peter (377) in 1900. His results were similar to those reported by other investigators for other crops. Woodman and associates (551) studied the chemical composition of pasture grass on a dry basis. The protein varied from 20.6 to 26.2 per cent, ether extract from 3.95 to 6.41 per cent, nitrogen-free extractives from 35.2 to 50.6 per cent and crude fiber 10.9 to 17.7 per cent. These results were amply confirmed over a period of years (539, 541, 545, 549, 550, 551, 552, 116). The composition of grasses with development was also determined by other investigators (40, 108, 225, 375, 133). Similar results were reported for cereal grasses (446).

The seasonal variation in the tannin content of *Lespedeza sericea* showed that the tannin increased to about the middle of June and then leveled off. Most of the tannin was in the leaves (72). The authors regarded the tannin content of plants as a factor affecting palatability.

The chemical composition of many grasses at the before heading, full bloom and seed stages was determined (239). Timothy contained 12.8 per cent protein before heading and 5.9 per cent at full bloom stages of maturity. The crude fiber showed an inverse relationship to that of protein. Similar results were reported for brome grass, slender wheat grass, western wheat grass and crested wheat grass. The average total sugar content for these grasses before heading, bloom and seed stages was 10.5, 6.2 and 3.9 per cent on a dry basis respectively.

These workers also reported the sugar content of grasses on the dry basis as dextrose. The results are shown in Table 3.

TABLE 3

Sugar content of grasses (calculated on dry basis as dextrose)

	Total sugars			Reducing sugars		
	Before heading	In bloom	Seeds ripe	Before heading	In bloom	Seeds ripe
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brome	11.07	7.98	4.22	6.67	3.65	2.06
Timothy	13.56	7.77	3.80	8.52	5.60	1.49
Western wheat	8.22	4.14	4.83	5.35	2.41	1.50
Slender wheat	9.30	4.73	2.87	6.84	3.49	1.34
Average	10.54	6.16	3.93	6.84	3.79	1.60

This phase was studied because the high sugar content of roughages is sometimes associated with palatability. The high quality of hay made from immature plants may be associated with the increased sugar content. These workers stated, however, that because of the solubility and ease with which it is fermented much of the sugar may be lost in the curing of grasses for hay under unfavorable conditions.

Capen and LeClerc (65) in Alaska studied the change in composition of native sedge, red top and cottongrass at various stages of maturity. The protein and total sugar in the dry matter decreased with maturity while the crude fiber increased. The decrease in sugar did not occur until rather late in the season.

Phillip and Goss (383) determined the composition of barley plants at different stages of growth in oven-dried material. The results are shown in Table 4.

After an initial increase, the percentage of ash and nitrogen declined

TABLE 4

Composition of barley plants (dry basis)

Age in days	Ash	Protein	Pentosans	Cellulose	Lignin
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
7	13.68		9.0		1.48
21	16.48	38.0	9.3	19.0	2.31
35	14.20	24.1	11.5	21.9	2.88
49	10.12	12.2	12.8	28.6	5.10
63	8.66	9.9	19.2	28.7	6.80
77	7.77	6.1	22.8	30.3	6.97
86	7.49	3.7	23.9	31.6	7.74

steadily as the plant matured. The percentage of cellulose increased rapidly as the plants grew older. The percentage of lignin, as well as the methoxyl in the lignin, increased in a regular manner as the plants developed and matured. The lignin in young plants differed from the lignin of mature plants in that the former contained a much smaller percentage of methoxyl. As the plants matured not only did the percentage of lignin increase but there was also a rapid methylation of the hydroxyl groups of the lignin. In the mature plants, 75 to 80 per cent of the firmly bound methoxyl groups were found in the lignin. This paper gives references to the early work on the subject.

The chemical changes which take place in rye grass with maturity were studied by Norman (368). Cellulose and lignin increased markedly as the plant approached maturity. Protein, pectin and fructosan decreased as the plant matured but the furfural from hemicelluloses did not exhibit a definite trend. A peak of 30 per cent of levulosans during early development was an interesting observation.

The chemical composition of the alfalfa plant at various stages of maturity was investigated by Widtsoe in 1897 (517). In the spring when the alfalfa was 6.5 inches high, the protein and fiber were 28.0 and 12.3 per cent respectively, on the dry basis. At the early bloom stage these values were 16.3 and 30.5 per cent and in late bloom they were 10.7 and 42.3 per cent respectively. The chemical composition of the second and third cuttings were determined in a like manner. Widtsoe also studied the variation in the chemical composition of leaves and stems of alfalfa. Continuing this investigation, Widtsoe and Stewart (518) reported that the water soluble constituents in early, flowering and later periods for the first crop were 21.9, 17.6 and 11.3 per cent respectively. The hexose, hexobiose, dextrin, starch, pentosans, crude fiber, ether extract, and protein fractions at various stages throughout the season were determined. There was a decrease in sugars, dextrin and protein fractions with maturity. Starch, pentosans and ether extract showed no significant trends but the crude fiber increased markedly.

The effect of maturity on the chemical composition of alfalfa was shown by Dinsmore in 1907 (104). The results were similar to those reported by

TABLE 5

Composition and feeding value of pasture grass and alfalfa cut at different stages of maturity (dry basis) compared with cereal grains (306, 350, 540, 550, 552, 549, 547, 542, 548)

	Protein	Co-ef. of dig. of prot.	Dig. protein	Fiber	Co ef. of dig. fiber	N. F. E.	Co ef. of dig. N. F. E.	Co ef. of dig. org. mat.	Starch value
	per cent		*per cent*	*per cent*		*per cent*			
Pasture									
Cut weekly	24.7	81	20.0	15.4	76.7	44.79	81.5	79	67.7
" every 2 weeks	23.5	80	18.8	15.9	81.3	44.53	82.8	80	69.8
" " 3 weeks	21.1	79	16.7	17.2	79.2	46.68	83.5	80	69.4
" " 4 weeks	19.4	76	14.7	21.2	81.7	46.27	82.8	80	67.1
" " 5 weeks	18.3	74	13.6	21.9	82.1	47.18	79.1	77	64.7
Good meadow hay	13.8	63	8.7	25.8				65	45.2
Poor meadow hay	7.5	45	3.2	33.5				52	24.0
Alfalfa									
Prebud	25.3	84	21.3	22.1	63.7	38.13	79.8	76	59.8
In bud	20.4	76	15.5	23.9	46.3	42.22	74.2	66	50.8
In flower	17.2	74	13.0	29.7	42.6	39.35	70.2	60	42.4
Average alfalfa	14.2	74	10.5	29.5	50.8	40.91	73.7		33.0
Cereal Grains									
Oats	12.4	78	9.7	10.9				70	64.0
Barley	11.5	78	9.0	4.6				88	74.0
Corn	9.6	74	7.1	1.9				9	84.0

Widtsoe and Stewart (518) for protein, ether extract and crude fiber, except that the variations were more pronounced, due to the fact that the samples were collected earlier in the season. Similar results were reported by Woodman and associates (547). The chemical composition of legumes at various stages of maturity has been reported by Sotola (443). Hunt and associates (251) also showed the effect of the stage of maturity on the chemical composition of alfalfa hay. Several other investigators have studied the chemical composition of alfalfa cut from bud to seed stages of maturity (420, 174, 272, 443). The results likewise show that the protein decreased and the fiber increased with maturity. Most investigations indicate that the greatest yields of dry matter and protein are secured with alfalfa when cut in the one-tenth to one-half bloom stage (420, 174, 273).

The changes in composition, digestibility and starch values of grass and alfalfa cut at various stages of maturity are shown in Table 5. The coefficient of digestibility of the organic matter of pasture grass cut every five weeks was 77 per cent compared with good meadow hay with a coefficient of 65 per cent. The data indicate that alfalfa cut in the bud stage has a much lower coefficient of digestibility than that cut in the prebud stage. This finding indicates that there is very little to be gained by cutting alfalfa before the one-tenth bloom stage, especially in view of the greater yield of dry matter and digestible protein per acre and less danger to the stand due to later cutting.

Hilton and associates (227) reported the yield of hay, dry matter, milk and butterfat per acre for early and late cut soybean hay. The results are presented in Table 6.

TABLE 6

Early versus late cut soybean hay for dairy cows

	Hay per acre tons	Dry matter per acre tons	Milk produced per acre pounds	Butterfat produced per acre pounds
Early cut*	1.614[1]	1.470	2,058	71.56
Late cut**	1.794[1]	1.608	2,451	92.62

[1] Yield on air dry basis.

* Cut when most of pods had formed and the lower pods were beginning to fill.

** Cut when pods were completely formed, the beans had practically reached their full development.

In this experiment relatively late cut soybean hay gave greater yields of hay, milk and butterfat per acre than early cut hay. As the soybean plant approached maturity it is apparent that the decrease in the percentage of protein in the leaves was more than compensated for by the increase in the percentage of protein in the pods.

The maximum quantities of total and digestible protein are present in timothy hay at about the full bloom stage of maturity. The digestible protein in relation to total protein declined rapidly after full bloom (471).

The composition of Sudan grass at successive stages of growth was investigated by Wright and Shaw (560) and is presented in Table 7.

TABLE 7

Composition of Sudan grass at successive stages of growth (dry basis)

Stage of maturity	Dry matter	Protein	Ether extract	Crude fiber	Reducing sugar	Non-reducing sugar	N. F. E.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
2 ft. high & under	15.5	20.2	2.6	24.2	7.3	0.7	45.7
3–4 ft. high	13.9	15.5	2.4	26.0	11.3	2.1	48.7
4–6 ft. high	20.0	8.3	1.5	29.8	17.4	2.8	56.0
Early bloom stage	22.3	8.1	1.2	30.6	16.0	1.1	56.2
Middle bloom stage	34.2	5.0	1.1	33.7	11.0	1.5	58.8
Early dough stage	36.9	4.8	1.1	33.7	10.2	0.7	56.2
Advanced dough stage	37.7	4.8	1.3	33.4	10.3	0.3	55.8
Seed stage	41.3	5.3	1.2	33.4	5.6	0.3	55.4

The high protein content of the young plant, 3 to 4 feet tall, together with the crude fiber content suggests the reason for the popularity of Sudan grass for pasture.

The prussic acid content of young well fertilized Sudan plants may be sufficiently high to be toxic to cattle (49). This objection can be overcome by waiting until the plant is at least 18 inches high before turning cows on to it. There appears to be little danger from pasturing Sudan grass when the cows are in good condition. Leaves and young parts of sorghum plants are higher in hydrocyanic acid than the other parts of the plant. More mature parts are also lower in this respect (309).

The total dry matter yield of a few varieties of corn determined at various stages throughout the growing season showed that most of the dry matter production per acre is produced during the last few weeks of the growing season (1). The best variety of corn to use for silage is one which utilizes the growing season to the best advantage in the production of dry matter but at the same time reaches the dough stage of maturity most years.

Relation of Rainfall to the Composition of Roughages.—Alfalfa grown in a humid area generally has a higher phosphorus content than that grown in an arid area (482). The amount and distribution of rainfall are factors of paramount importance which influence the yield of both dry matter and protein (435, 8, 123, 109, 91). Midgley (331) concluded that since moisture is a limiting factor in the production of good pasture, it is evident that soil type has a marked effect on the quality and quantity of roughage. During dry seasons the phosphorus content of herbage cut at monthly intervals tends to be low and the calcium content tends to be high (109). Other investigators have also observed a decrease in the phosphorus content of the herbage due to insufficient rainfall (111, 142). Severe outbreaks of malnutrition among cattle following a drought have been associated with insufficient phosphorus intake (474, 19, 464, 111).

Relation of Soil to Composition of Roughages.—Lawes and Gilbert (293) observed that the mineral content of forage plants is subject to wide variations as a result of soil conditions. Some of the early work which showed the relationship of the mineral content of feeds to the moisture content of the soil was reviewed by Eckles and associates (111) and Orr (371).

An application of complete fertilizer followed by later applications of nitrogenous fertilizer on a rotationally grazed pasture decreased considerably the percentage of dry matter in the grass and decreased slightly the percentage of crude fiber and calcium in the dry matter (11). The percentage of nitrogen, phosphorus and ether extract was increased, the nitrogen markedly so.

In most cases the application of 100 to 250 pounds of superphosphate per acre did not materially influence the phosphorus content of the dry matter (47). In some cases, however, the increase was as much as 40 per cent. Application of phosphorus to mixed herbage plots tended to increase the percentage of phosphorus in the hay. This may have been due to changes in type of vegetation as a result of phosphorus treatment. The phosphorus content of the grass was increased to an appreciable extent by phosphorus fertilizer (352). Phosphorus fertilizer increased the phosphorus content of white clover 10 per cent and Kentucky bluegrass 40 per cent. Calcium fertilizer added to acid plots increased the calcium content of brome sedge 19 per cent and Kentucky blue grass 36 per cent. Similar results were reported by other investigators (24, 483).

The use of manure or phosphorus fertilizer on alfalfa grown on a calcareous soil, high in total but low in available phosphorus, gave increased yields of hay. There was also a marked increase in the phosphorus content (388).

Fertilization increases the protein and phosphorus content of the pasture herbage (448, 295). The treament of pasture with large amounts of nitrogen and smaller amounts of superphosphates and potash gave outstanding production of herbage (53).

The application of a phosphate fertilizer increased the phosphorus content of grass, oats, and soybeans (341). Mather (310) reported that an application of 200 pounds of treble superphosphate was sufficient to give maximum yields in pounds of dry matter but that much larger applications were necessary to increase the phosphorus content significantly. He concluded that phosphate fertilizer could be profitably applied only insofar as it increased the pounds of hay per acre, and that the phosphorus required in a ration in excess of the quantity supplied in such a hay could be added directly to the feed.

Bone-eating habit in cattle was traced to phosphorus deficiency in the hays and grasses eaten and was found to be confined largely to swampy areas, the grasses of which were shown to be much lower in phosphorus than the upland grasses (55). Triple superphosphate applied at the rate of 200 pounds per acre increased the yield of first cutting alfalfa by three per cent and increased the phosphorus content 10 4 per cent. The second cutting was increased 3.1 per cent in yield and 6.4 per cent in phosphorus content. Similar results were reported by other investigators (56, 5).

Jones and associates (260) studied the effect of fertilizing irrigated pastures. The addition of 300 pounds of 16 per cent superphosphate per acre increased the yield of dry matter 85 per cent. There was no effect on the calcium and phosphorus content of the herbage. The yield or composition of bench land and alluvial land pastures were not affected by nitrogen and phosphorus fertilizers (303). In an earlier experiment, Lush (304) reported that the addition of fertilizer to pasture resulted in a greater growth rather than effecting the composition of plants. Milk production from nitrate and phosphate pasture was doubled; and from cyanamid, trebled over unfertilized pasture. Nitrogen fertilizers increase the protein and decrease the fiber content of plants (130).

The average protein content of rotationally grazed pasture was increased from 22.6 to 25.3 per cent by intensive fertilization with nitrogen fertilizers (175). Enlow and Coleman (125) concluded that the protein content of pasture in a grazed condition can be increased and maintained at a somewhat higher level than ordinary by frequent light applications of nitrogen fertilizer.

Fertilizers improved the quantity and quality of the grasses but nitrogen fertilizer had an immediate pronounced effect (137). The cumulative effect of fertilizer is an outstanding factor. This system of pasture management lengthened the grazing season and increased the carrying capacity thus reducing the amount of barn feeding. The increase in the yield of dry matter was a more important result of the fertilizer treatment than any increase in the percentage of protein, calcium or phosphorus (180). The percentage of crude fiber was consistently higher in the fertilized crops.

Shutt and Hamilton (435) also reported that nitrogen fertilization increased the yields of dry matter and protein. Beaumont (30) increased the calcium, nitrogen and phosphorus in pasture grass by fertilization and the fertilized pasture grass was found to be more palatable. The fertilization of quack grass resulted in an increased growth associated with high protein content (99). The fertilization of blue grass increased the protein in the early part of the season but in early June the effect was slight (377).

Rabbits fed fertilized grass made good gains while those fed unfertilized grass made poor gains (81). The difference could not be detected by chemical analysis. The literature dealing on this subject was reviewed by Crampton (78).

Pratt and Holdaway (395) applied fertilizer to permanent blue grass sod which resulted in an increased milk production ranging from 31 per cent for the annual application of an incomplete fertilizer to 75 per cent for heavy applications of complete fertilizer. The fertilizer cost increased the cost of the total digestible nutrients. Crampton and Raymond (83) as a result of four years work with steers, stated that their results showed that fertilization practically doubled the carrying capacity of pastures. The protein, calcium, and phosphorus content of the herbage was also increased. These investigators stated that the nature of the protein is in some way changed by fertilization so that more rapid gains are obtained per unit of feed eaten.

Phosphate fertilization increased the white clover (52). The carrying capacity and the gains of the cattle paralleled the changes. Based on the unfertilized plot, 500 pounds of superphosphate produced an average increase of 64 per cent, while one ton of limestone together with superphosphate gave an additional increase of 50 per cent.

Soil fertility apparently plays an important role in the carotene content of plants. Guthrie (188) working with soybean plants, showed that an insufficient supply of nitrate nitrogen adversely affected the amount of both carotenoids and chlorophyll present. Virtanen and co-workers (488) showed that carotene is directly related to the facility with which the plant can utilize a given nitrogen supply and that nitrates are superior to ammonium salts. Furthermore, a maximum carotene content was reached when plants were grown at an optimum pH, which for wheat and peas, was assumed to be 6.5.

Thomas and Moon (467) investigated the carotene content of pasture grass and yield of carotene per acre as influenced by different fertilizer treatments. Sulphate of ammonia applied at monthly intervals produced marked increase in carotene content, yield of dry matter and total yield of carotene. Sulphate of iron applied monthly at a low rate, was without effect on carotene but seriously reduced yields of dry matter, while applications of $CaCO_3$ had no appreciable effect. There was a correlation between the carotene content of grass and the protein. A majority of the samples had a carotene-xanthophyll ratio of approximately 1 to 2.5. Nitrogen fertilizer increased and potassium fertilizer decreased the carotene content of plants (253).

Relation of Soil Type to the Chemical Composition of Roughages.—Grizzard (179) showed that chemical composition and the effect of fertilizer on alfalfa depends on the soil type. In general, the plants grown on soils not requiring applications of limestones showed increases in the phosphorus content of both stems and leaves with fertilizer treatments containing superphosphate alone or with potash. Plants grown on Isabella type of soil showed that all soil treatments containing limestone increased the nitrogen but tended to decrease the phosphorus content of alfalfa.

Nitrogen, phosphorus and potassium fertilizer applied alone or in combination to the soils in Western Washington had no appreciable effect on the percentage of these elements in the alfalfa hay in the majority of cases but when applied to Eastern Washington soils the phosphorus and potassium content of the hay had a tendency to increase as a result of phosphate and potash fertilization (482). Similar results were reported by Grunder (180).

The phosphorus content of alfalfa hay grown on heavy soils was higher than that of hay grown on light soils (332). Superphosphate applications did not change the phosphorus content of field-grown alfalfa hay. The phosphorus content of field-grown red clover and sweet clover was increased by superphosphate fertilization. Limestone applications reduced the phosphorus content of alfalfa.

Plants are materially affected by the mixture of the soil on which they are grown (331). If the soils are high in available plant nutrients, it will be reflected in the chemical composition of the plants and likewise in the performance and physical condition of the grazing animals. Soil type greatly affects the kind of plant it will support. If sufficient moisture and minerals are present, more clover will be produced than when these essentials are lacking. Since legumes contain more protein and minerals than grasses, it is evident that soils indirectly affect the chemical composition of the herbage by influencing the botanical composition.

The effect of the application of phosphatic fertilizer to several different types of soil on the phosphorus content of plants gave varied results (120). On some soils the application of phosphorus resulted in increased phosphorus content of the plants while on other soils no response was observed. The authors suggested that the phosphate content of hay may be increased by the application of phosphate fertilizers if the phosphate in the soil is either low in quantity or not readily available.

More crops respond to a nitrogen fertilizer than to fertilization by any other element. This is especially true of the grasses used for pasture and hay. The application of phosphorus to phosphorus deficient soils results in greater yields of dry matter but does not always increase the phosphorus content of the pasture or hay crop. The per cent of phosphorus in such crops is influenced by the soil type, amount of moisture, amount of available phosphorus, and probably by many other factors. In certain sections where the application of phosphorus in quantities sufficient for maximum dry matter production does not materially increase the percent of phosphorus, the feeding of a phosphorus supplement is indicated when a protein concentrate rich in phosphorus is not fed.

Greater returns can usually be secured by fertilizing hay crops rather than pasture. The fertilization of pastures frequently results in a peak production in the spring but does not aid in solving the summer pasture problem when the temperature is high and the rainfall is low. This may not be true for regions which have sufficient summer rainfall.

PRESERVATION OF ROUGHAGES

In regions where good pasture is not available all the year round the preservation of roughages becomes important. Grasses and legumes have usually been preserved as hay while such roughages as corn, sorghums and sunflowers have been conserved as sun-cured roughage and as silage. Unfortunately there are many sections where it is impossible to make good hay due to unfavorable weather conditions.

Losses in Field Curing and Storage of Hay.—Wolff (192) in 1895 found that 20 per cent of the dry substance of hay may be lost by simply soaking in cold water and that clover hay suffered from rain more than meadow hay because from 25 to 40 per cent of its dry matter may be dissolved. He reported analyses by Ritthause at Möckern, Germany, on two samples of clover hay which were cut at the beginning of the flowering period from the same field at the same time. One was quickly dried and the other left for two weeks exposed to intermittent rains. The results are given in Table 8.

TABLE 8

Effect of rain upon the composition of hay

	Water	Crude protein	Nitrogen-free extract	Crude fiber	Ash
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Not rained on	16.0	14.6	36.1	25.3	8.0
Rained on	16.0	15.6	23.4	37.4	7.5

Headden (209) in 1896 reported the changes in the chemical composition of alfalfa hay damaged by rain. The results are given in Table 9.

TABLE 9

Effect of rain on the composition of hay

	Alfalfa dried in air bath Dry basis	Alfalfa in field May 28 to June 12, rainfall 1.76″ Dry basis
	per cent	*per cent*
Ash	12.18	12.71
Crude fiber	26.46	38.83
Crude fat	3.94	3.81
Crude protein	18.71	11.01
Nitrogen-free extract	38.71	33.64

The weather during this time was cloudy and the temperature ranged from 72° to 81° F. The loss due to weathering as shown in table 9 is probably greater than usually occurs due to the fact that the hay which was not rained on was not field cured. Headden mentioned the mechanical loss of leaves and stems but attributed the changes in composition to the action of heat and moisture.

A sample of greenhouse barley was leached by soaking in water for several minutes. The losses were as follows: protein, 1.6 per cent; phosphorus, 36 per cent; potassium, 65 per cent; sodium, 52 per cent; magnesium, 45 per cent; and chlorine, 75 per cent. Very little leaching occurred in the plant until drying had taken place, whereas, if the rains came after the hay has been dried, the loss was considerable (294).

Westgate (514) sprinkled perfectly cured crimson clover hay with water to imitate rain for one hour on each of three successive days. The sprinkled sample lost about three-fourths of its sugar, one-ninth of its protein, and three-fourths of its ash. Rain washes out nitrogen-free extract; sugar, a constituent of the nitrogen-free extract; and amides (436). The weathering of clover, which included exposure to rain, resulted in a decrease in digestibility of each constituent except crude fiber (185). The loss of soluble constituents caused by rain may have been responsible for a greater part of the decreased digestibility. Bleaching and leaching apparently decreased the palatability of the bur clover. The effect of leaching on the chemical analyses was also studied (186). Loss of silica-free ash, 26 to 27 per cent; chlorine, 67 per cent in oat hay and 86 per cent in bur clover. Phosphorus loss was appreciable; nitrogen-free extract loss varied from 6 to 35 per cent; crude protein loss varied from 1 to 16 per cent, usually not very significant; ether extract was not affected and fiber increased.

Wiegner (519) working in Switzerland studied the losses due to hay making. The results are presented in Table 10.

TABLE 10

Losses of nutrients in hay making

	Percentage losses	
	Digestible protein	Starch equivalent
Dried indoors, no rain, no mechanical losses	13.8	22.6
Dried outdoors, no rain, leaf shedding	32.7	38.6
1–2 showers of rain	27.8	43.6
5–6 showers of rain	49.8	54.2
Average of all experiments in which it rained	40.4	49.7

Losses of starch equivalent and digestible true protein increased with the amount of rain falling on the crop in the field. In an extreme case, a loss of 62 per cent of the starch equivalent of the fresh crop and 45 per cent of the digestible true protein was reported (84).

The data in Table 11 were compiled by Watson and associates (511) and show the losses of dry matter, starch equivalent and protein equivalent in hay making.

The hay was meadow hay in all cases. The large losses of starch equivalent and protein appear significant.

TABLE 11

Losses arising during hay making with field meadow grass

Year	Weather conditions	Type of hay	Percentage of the original material		
			Dry matter	Crude protein	Starch equivalent
1932	excellent	early hay	20.5	17.6	31.4
	poor showers	ordinary	33.9	51.3	46.7
1933	fair	early hay	15.5	12.7	36.1
	excellent	ordinary	12.2	11.5	25.6
1934	very good	early hay	5.6	6.8	19.4
	excellent	ordinary	10.0	9.0	21.7
1935	very poor	early hay	30.5	20.2	51.2
	good	ordinary	1.2	2.4	12.6
Average		early hay	18.0	14.3	34.5
		ordinary	14.3	18.5	26.7

From a 2-year investigation in eight localities in Norway (412) it was found that the total loss of food nutrients was 20 per cent. Kiesselbach and Anderson (272) studied the effect of rain on the protein content of alfalfa hay excluding leaf loss The results are shown in Table 12

TABLE 12

Effect of rain on field cured hay

Number of comparisons	Exposure to rain	Hours required	Moisture content	Color	Quality leaves	Protein	Grade
			per cent	*per cent*	*per cent*	*per cent*	
Tests June 18							
14	no	74	28	48		16.9	2
14	yes	191	23	40		17.0	2
Tests July 11							
5	no	54	31	57	39.2	17.4	2
5	yes	136	31	32	39.2	18.6	3
Tests August 14							
2	no	44	17	65	38.0	16.4	1
2	yes	131	15	40	38.0	16.8	
Averages							
21	no	57	25	57	38.0	17.1	2
21	yes	153	23	37	37.9	16.5	2

Alfalfa dried in the sun had a higher total protein content that that dried in the shade (458). The loss in digestible constituents during drying in the field may vary from 20 per cent under favorable conditions to 50 per cent under adverse weather conditions (433). Sweet clover dried in the sun had a higher total protein content than hay that was dried in the shade (222). Alfalfa samples which were dried in the sun had a larger percentage of water soluble carbohydrate than those dried in the shade. Sprinkling decreased the water soluble carbohydrate for both the samples dried in the shade and those dried in the sun. Weiske (512) noted that meadow hay held at a temperature of 100° F. was lower in digestible protein than that kept at atmospheric temperature even when kept at this temperature for two to four weeks. This change was somewhat more marked on the moistened hay both in chemical composition and in palatability.

Fleischmann (136) found that under the most favorable conditions of curing there may be no loss of dry matter. If the drying is not too rapid in uninterrupted sunshine there may be an actual gain in weight which may be ascribed to assimilation. Under less favorable conditions losses may occur and under very unfavorable conditions the loss of dry matter in harvesting may exceed 20 per cent.

A comparison of alfalfa and red clover hay dried in an oven, in the sun, and in the shade indicated that under such conditions fermentation does not take place to any great extent (222). Paturel (374) showed a loss of 15 to 35 per cent in dry matter of damaged alfalfa, clover and common hay. A feeding trial indicated a proportionate loss in feeding value. Cooke (76) found as an average of eight tests in Colorado that the loss in dry matter under ordinary methods of field handling varied from 5.1 to 26.1 per cent. The extreme values represented differences in weather conditions. Kenney (269) stated that the average loss in harvesting 41 lots of alfalfa in Kansas in 1914 was 12.43 per cent of the entire crop. The loss of leaves varied from 6 to 48 per cent.

Mechanical Losses.—The loss due to mechanical causes is a variable one. The shedding of leaves is the most serious due to the fact that the leaf is high in nutritive value. Salmon and associates (420) determined the leaf loss in harvesting alfalfa from 1914 to 1920. The results are presented in Table 13.

TABLE 13

Leaf loss in harvesting alfalfa hay

Stage of maturity	Per cent leaves	Per cent of total crop
Bud	19.1	10.1
1/10 bloom	14.5	7.3
Full bloom	20.4	10.1
Seed	22.2	9.3

Over this period 19 per cent of the leaves were lost which amounted to 9.2 per cent of the total crop. The leaf loss varied with the season ranging from 8.6 to 25 per cent of the leaves. When the conditions were unfavorable and the hay was handled carelessly, half of the total weight of the alfalfa and a much larger part of the feeding value was lost (511).

The plant when cut for hay is still alive and until the cell is killed as a result of drying, it still continues to breathe. The act of breathing causes a loss of carbohydrates. Further, though less serious, losses are due to the action of enzymes.

TABLE 14

Losses in hay making (*519*)

	Dry matter	Digestible dry matter	Starch equivalent
	per cent	*per cent*	*per cent*
Respiration	up to 10	5–15	5–15
Mechanical losses	5–10	5–10	5–10
Fermentation in the stack	5–10	5–10	5–10
Losses due to extra energy necessary to digest hay			10–15
Total	10–30	15–35	25–50

Newlander and associates (363) studied the percentages of the various nutrients lost or gained by different methods of conserving green alfalfa and timothy for winter use. The results are shown in Table 15.

TABLE 15

Nutrients lost or gained by different methods

	No. of tests	Dry matter	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Silage*	24	− 8	− 5	− 7	− 1	−15	+17
Sun-cured hay	4	−15	−21	−21	−12	−14	−28
Artificially dried hay	4	− 5	−10	−17	0	− 3	− 5

* Losses due to spoilage at top of silo are not included.

Watson (506) recently compiled the percentage losses in roughage conservation as shown in Table 16.

TABLE 16

Percentage losses incurred in conservation processes

Process	No. of tests	Dry matter	Starch equivalent	Digestible protein
		per cent	*per cent*	*per cent*
Hay making	39	22.1	43.9	34.6
Hay making with special appliances	96	17.4	38.2	25.3
Artificial drying	2	7.5	11.5	8.2
Ordinary silage	225	15.9	34.3	39.8
Stimuated (molasses)	34	11.7	22.9	10.0
Acidified silage	74	12.4	23.7	10.0

The time required for hay to reach 30 per cent moisture and the percentages of dry matter recovered by different methods of sun-curing were studied by Kiesselbach and Anderson (273). Hay cured in the swath throughout reached a moisture content of 30 per cent in 27 hours but lost

18.5 per cent of the dry matter. Hay which was cured in the swath for two hours and then cocked reached 30 per cent moisture in 32 hours and lost only two per cent of its dry matter. The data indicated that the most practical method was to cure for three hours in the swath and then put in a windrow. By this method 30 per cent moisture content was reached in 27 hours with a loss of only 1.7 per cent of the dry matter.

The size of the windrow affected the length of time required for hay to cure but did not affect the quality of the resulting hay (272). Also, hay windrowed 4, 8 or 24 hours after cutting, cured more rapidly than the hay which was raked immediately after cutting but not as rapidly as that cured in the swath. Partially swath cured hay graded higher than the hay from either of the other methods of curing. Higgins (222) emphasized the importance of curing hay so as to have the leaves and stems remain soft and tough.

Truninger (473) followed seven lots of hay in detail from the time of cutting to the time of storing. There were changes in dry matter ranging from a maximum loss of 11.3 per cent to a maximum gain of 1.3 per cent. The storage of these lots of hay in small stacks whose moisture content ranged from 23.4 to 39.1 per cent, resulted in further loss of 2 to 11 per cent or an average of six per cent for the seven lots. The chief loss occurred in the nitrogen-free extract, the changes in these constituents ranged from a loss of 40 per cent to a slight gain in two cases. Crude protein remained practically unchanged although there was a marked decrease in the coefficient of digestibility of the pure protein.

Losses in Storage.—Browning of hay resulted in a marked decrease in the digestibility of protein (237). Swanson and co-workers (457) studied the losses in making brown and black alfalfa hay by stacking partially wilted alfalfa having an average moisture content of about 40 per cent. The losses in protein, crude fiber, nitrogen-free extract, and ether extract were 32.7, 46.5, 45.4, and 63.6 per cent respectively. Black alfalfa produced as a result of fermentation was found to be quite inferior as a feed for steers to brown alfalfa or alfalfa of good quality and color. Steers on black alfalfa made an average gain of 1.45 pounds per day compared with 1.94 and 1.96 pounds respectively for brown and regular alfalfa hay.

This problem was recently studied by Hoffman and Bradshaw (235) in a series of seven large scale storage experiments. The samples of hay investigated contained 28 to 70 per cent moisture. The loss of organic matter ranged from 4 to 22 per cent with an average of 13 per cent. The loss varied with the maximum temperature which did not exceed 78° C. in this work.

The losses in crude fat ranged from 6 to 47 per cent. The loss of crude fat was accompanied by complete loss of carotene. The sugars suffered a severe loss and in several cases they were almost entirely destroyed. The losses ranged from 59.1 to 93:7 per cent indicating a very high degree of active fermentation. Hemicelluloses as measured by the pentosan value underwent a loss of 13.9 to 52.1 per cent as determined by hydrolysis. Cellulose showed a loss ranging from 6.7 to 21.1 per cent in two experiments. Some samples showed a slight gain. Lignin showed gains, except in three samples, in which very small losses were recorded.

Spontaneous combustion of hay was attributed to the presence of pyrophoric iron produced by cellulose. Hay thoroughly exposed to the sun's rays must be richer in hydrocarbons is advanced as the reason why more stacks are destroyed by fire during a good hay season (292). Hay stack fires occur more frequently during a year of good conditions for hay making. The literature on this subject has been reviewed recently (565).

Woodward and Shepherd (557) studied the loss of dry matter during storage of hay finely chopped (one-fourth inch), coarsely chopped (three-fourths inch), and long alfalfa hays. These hays contained from 25 to 27 per cent moisture when stored. Approximately three times as much finely chopped and twice as much coarsely chopped hay could be put in a given space as long hay. The chopped hays lost all traces of green color during storage, whereas considerable of the green color was retained in the long hay. The carotene loss was extensive in all lots but somewhat greater in the long hays. The loss of dry matter during storage was highest (6.5 per cent) in the finely chopped lot. The long hay was more efficient than the chopped hays in tests with milking cows. These workers (432) also found that hay with 16 per cent moisture lost more green color and carotene when stored in a chopped form (three-fourths inch) than when stored in long form. There was no significant difference in milk production when chopped or whole hay was fed. The cows consumed slightly more of the chopped hay.

Hay of a distinct brown color did not result if the moisture content of the hay as stored did not exceed approximately 30 per cent (413). The maximum loss of dry matter was 22 and the average loss 14 per cent. Losses in organic matter included mostly the fats, sugars, and hemicelluloses. Protein and cellulose were lost under more destructive conditions.

Effect of Salt on the Microbial Heating of Alfalfa Hay.—According to Hill and White (223) the fact that sodium chloride inhibits the growth of certain microorganisms is well known. Shutt (434) observed that in every case hay to which salt was added during the storing process appeared brighter than hay stored unsalted. No appreciable chemical difference was noted. Feeding experiments indicated that 20 pounds of salt could be added per ton without any bad effects but when 30 pounds per ton were added bad physiological effects resulted.

Stuart and James (454) after studying the effect of adding one, two and five per cent salt to hay, concluded that salt added to moistened alfalfa hay will inhibit bacterial growth and will delay but not prevent mold development. This delay in microbial development may be long enough to permit the curing of hay. The use of 1.5 per cent of salt added to long alfalfa hay averaging 35 per cent moisture had no appreciable effect in preventing or retarding spontaneous heating (413). Hartwig (201) reported that hay ranging in moisture content from 20 to as high as 40 per cent was not benefited by the addition of 12 to 24 pounds of salt per ton. The age old practice of salting hay does not appear justified from the standpoint of hay quality (413, 565).

Artificial Drying of Hay.—It is apparent that the loss of nutrients during hay making under ordinary conditions is rather great. This fact has stimulated an interest in methods of artificially curing roughages. A method of artificial drying was devised in 1894 by an English farmer named Neilson (385). By this method the green material was placed in rough stacks having a cylindrical open space at the center extending from the bottom to near the top. A pipe extending under the stack connected this open space to a powerful fan which sucked out the air, thus causing the outside air to penetrate the stack. The fan was operated from 15 to 60 minutes daily for from one to three weeks.

The Institute of Agricultural Engineering of the University of Oxford conducted a series of experiments on the artificial drying of hay crops in 1925 (254) by driving air with a fan through a tunnel into an open space under the stack of green material. Peas, beans and cereals were successfully dried by using air of atmospheric temperature. The conclusion was, however, that this method could not be depended upon under adverse weather conditions.

Recently Wylie and co-workers (561) left the hay in the field for a part of the first day until the hay contained about 45 per cent moisture. It was then stored in a loft equipped with a system of air ducts through which the air from a blower was forced upward through the hay or drawn down through the hay as desired. This process gradually removed the excess moisture and kept the hay at about the wet-bulb temperature. By blowing air through the hay during the day only, and allowing the excess moisture in the hay to move to the surface of the stems and leaves over night, curing was completed about as soon as by continuous blowing. The advantages claimed for this system are its cheapness and improved quality of hay.

In 1924 and 1925 the University of Oxford (254) dried hay with heated air. A temperature of 180° to 190° F. was not considered too high. Chemical analysis showed the feeding value of the hay dried artificially at these temperatures to be practically the same as that of the green plants and superior to that of hay dried under ordinary field conditions.

The data of Woodman and associates (539) and Watson (502) indicate that artificially dried young grass is in the class of concentrate food stuffs. Watson (502) also carried out an experiment using five cows in which artificially dried grass replaced grain in the ration for a period of 14 days. The results indicated that the grass was about equal to the concentrates for milk production.

Digestion trials with dairy cows fed solely on artificially dried young grass and on fresh green young grass, showed on the basis of 90.2 per cent dry matter, that the total digestible nutrient contents of the dried grass were 64.47 per cent and of the green grass 63.46 per cent (364).

Newlander (360) from the results of a double reversal feeding trial with milking cows observed an increased milk and fat yield when artificially dried young grass was substituted for hay in the ration on total digestible nutrient basis. He concluded that at least average milk and fat production could be secured by feeding home-grown artificially dried young grass in lieu of purchased concentrates. The data indicated that milk production did not hold up as well when the concentrates were replaced by dried grass. Camburn (62) concluded that considering both milk production and body gain the total digestible nutrients in the dried grass were equal or slightly better than those in the grain mixture.

Hodgson and Knott (229) observed the following coefficients of digestibility for artificially dried grass by dairy heifers: dry matter, 67; crude protein, 75; nitrogen-free extract, 22; and crude fiber, 73 per cent. Artificially dried pasture herbage may be efficiently used for at least a part of the concentrate mixture of milking cows (277).

Newlander (362) reported the following digestible protein and total digestible nutrient contents of the following artificially dried roughages: corn silage, dried 3.73 and 67.7; corn silage, wet 4.57 and 68.24; oat hay, 10.5 and 70.57; Sudan grass, 12.44 and 65.25; soybean hay, 9.00 and 71.43; and millet, 8.05 and 67.96 respectively.

Watson and Ferguson (508) alternated two groups of milking cows between a ration of roots, hay and concentrates and a ration in which approximately eight pounds of artificially dried grass per cow daily replaced part of the concentrates, the two rations being adjusted so as to supply equal quantities of protein and starch equivalents. There were significant differences in milk and butterfat yields. The greater carotene intake in the dried grass resulted in a marked increase in yellow color of milk.

Newlander (361) determined the digestibility of artificially dried young Sudan grass. The dry matter, protein, fiber and nitrogen-free extract were 70, 64, 71, and 77 per cent digestible. The total digestible nutrient content on a dry basis was 58.9.

The nutrients of green alfalfa exposed to temperatures of 480° to 535° C. in a Koon type drier for 40 seconds were no less digestible than those of similar alfalfa dried in the field (197).

Watson (504) concluded that a feed of high digestibility and hence high feeding value could be produced at a drying temperature of 600° C. He estimated the loss of nutrients during dehydration at five per cent. Bartlett and associates (27) reported that biological values of protein of young grass sun-dried, artificially dried at 300° F. and at 170° F. were 52, 67, and 62 respectively.

The digestibility of the various constituents of green grass, sun-cured grass and grass dried at various temperatures was studied (231). A temperature of 400° F. depressed the digestibility of the protein, dry matter, and nitrogen-free extract. The various temperatures used lowered the color.

The effect of varying lengths of time grass was exposed to air at 160°, 180°, and 200° C. was also investigated (503). The exposure of the dried material to hot air reduced the carotene and digestibility of the protein markedly at 160° and 180° C. when the time was greater than 10 minutes. With a temperature of 200° C. the losses were marked at 10 minutes.

Five Jersey heifers were started on artificially dried roughage alone at 12 months of age (167). They maintained slightly more than normal gains between 12 and 18 months. In another experiment 16 Jersey cows made 17 lactation period records on rations restricted to artificially dried hay and pasture. Their average production calculated to mature equivalent basis, for an average lactation period of 361 days was 6,333 pounds of milk and 329 pounds of butterfat. These same cows fed liberal amounts of grain in addition to artificially dried hay and pasture produced an average of 9,656 pounds of milk and 527 pounds of fat. On the roughage ration the cows produced 66 per cent as much milk and 62 per cent as much butterfat as they produced under the register-of-merit conditions. During the time that they received hay without pasture they consumed an average of 28.6 pounds of hay per cow per day, or at the rate of 3.44 pounds of hay per 100 pounds of body weight. The artificially cured hay was very palatable. Artificially cured hay which has not been exposed to the sun is deficient in vitamin D (33).

The problem of grass drying has recently been reviewed by Roberts (412). The temperature of the inlet gases may be as high as 700° C., under which conditions drying is extremely rapid, without causing any serious impairment of quality or feeding value, provided the drier is operated in such a manner that the material is removed from contact with the hot gases as soon as it is dry (537). Furthermore, under satisfactory conditions of drying, most of the carotene of the fresh grass is retained in the dried product and the use of the latter in the winter rations of dairy cows results in the production of a yellow butter comparable to that produced when the cows are on grass in the summer. Artificially dried alfalfa and soybean hays contained considerably more carotene than the same grades of hays cured in the sun (226).

The nutrients in properly artificially cured hay are well preserved during the curing process but the loss of carotene during storage at high temperatures is of about the same order as sun-cured hay. The most important disadvantage of artificial hay-drying methods now in use is the high cost. Low temperature, high windage in dehydration of hays may reduce the cost somewhat.

Conserving Roughages in the Silo.—The large loss of nutrients in the field-curing of roughages, especially during inclement weather has focused attention on the use of the silo. In the past corn and the sorghums were the principal crops ensiled. The literature pertaining to corn silage has been reviewed by Nevens (358). The trend toward soil conserving crops has raised the question of placing the grasses and legumes in the silo.

Early History of Grass and Legume Silage.—In 1887, the Vermont Experiment Station analyzed a sample of rowen silage made by a farmer (493). Peas and oats, Hungarian millet, clover, peas-vetch-oats, cow peas and soybeans and rye were ensiled and their feeding value studied (494, 495, 496, 497).

In 1896 Headden (209) compared alfalfa, red clover and pea-vine silage from the standpoint of chemical analysis. The alfalfa silage samples contained 30 per cent dry matter. Headden stated "It will seldom be advantageous for the farmers of this country to make their crop into ensilage, but if they should choose to, the ensilaged product, as shown above, will compare favorably with a very good quality alfalfa hay."

In 1900 Cooke (76) experimented with two batches of alfalfa silage, one with alfalfa put in whole as cut in the field, and the other with alfalfa cut in quarter-inch lengths. The whole alfalfa showed a spoiled layer around the side nearly all the way down. The silage at the bottom and middle was excellent and was greedily eaten by cows and calves. The loss in total weight was 10.7 per cent. The cut alfalfa was weighted with stone. When put in it contained 33.0 per cent dry matter. The silo showed two inches of spoiled ensilage on top and one-half inch on the sides at the time of opening. The loss in dry matter was 10.0 per cent. Shaw (431) in 1900 referring to legumes for silage said, "These can all be preserved with more or less success in the silo, but when preserved without mixing with other crops, in instances too numerous to mention, the success attained has been only partial."

Withycombe (530) in 1901 reported as a result of an investigation and observations in Oregon that legumes make good silage (clover, vetch and alfalfa). He stated that alfalfa should be harvested in full bloom for silage.

Clark (73) in 1913 following three years of experimental work, reported that clover silage gave good results. According to Upson (478) in 1915, alfalfa silage does not keep well, probably on account of a deficiency of fermentable sugar which is needed to form sufficient acid. Alfalfa mixed with sorghum in different proportions gave good results. Good silage was also made from alfalfa to which corn meal was added at the time of ensiling.

Woll (531) in 1917, stated that alfalfa silage was used to a considerable extent with good results in California. The best alfalfa silage observed was made from alfalfa cut at the beginning of bloom. It was cut in the morning and left to dry in the field until noon. No water was added.

The most extensive early investigations with alfalfa silage were made by Reed and Fitch (400). They concluded that alfalfa alone makes a fair quality silage but that it should be fed within a few months as the quality deteriorates rapidly after the silage is several months old. The addition of material containing easily fermentable carbohydrates, such as corn meal, blackstrap molasses, or mixing with such crops as sorghum gave the silage better keeping quality and greater palatability. The cattle ate very little straight alfalfa silage. Alfalfa and ground corn were mixed 10 to 1 and the alfalfa and molasses 20 to 1. The alfalfa used was high in dry matter, ranging from 38 to 58 per cent.

According to Swanson and Tague (459) silage could be made from alfalfa alone if the absolute exclusion of air and retention of CO_2 were secured. They also reported that wilted alfalfa was more suitable than unwilted for making silage. Molasses was the most effective preservative for alfalfa silage. Germinated corn was better than sound corn.

Hunter (252) reported that alfalfa siloed alone underwent a typical silage fermentation which was caused by microorganisms practically identical with those from normal corn silage. When fermentable carbohydrate was added to the silo at the time of ensiling a good quality of silage was produced although bacterial flora was not changed. Alfalfa silage which was made with a fermentable carbohydrate supplement had a higher acidity. More amino nitrogen and more ammonia were formed in the alfalfa silage than in the alfalfa and carbohydrate silage which indicated that the carbohydrate was a protein sparer. The theory was advanced that proteolytic action which is responsible for the offensive odors characteristic of alfalfa silage was due in part at least to the inability of the acid producers to utilize protein as a source of energy in the absence of carbohydrates. Alfalfa ensiled alone or in combination with straw developed a typical acid fermentation and was unfit for feeding purposes (356).

The important factor which determines the keeping quality of alfalfa silage is the water content (110). For the best results, alfalfa should contain nearly 40 per cent of dry matter when put into the silo. The limits for good results seem to be between 30 and 50 per cent of dry matter. During a season of normal rainfall alfalfa will, as a rule, contain from 20 to 25 per cent of dry matter when in the stage usually cut for hay. During a period of abnormally low rainfall the dry matter may be as high as 40 per cent. In order that the plants may continue to grow and produce a good crop for the next cutting, it is necessary to cut the crop before it is sufficiently mature and to have the proper amount of dry matter to make good silage. For this reason, except when the crop is unusually dry and firm as a result of a deficiency of rainfall, it is advisable to allow the alfalfa to lie in the field a few hours after mowing in order to eliminate some of the excess moisture. The proper stage at which to put alfalfa into the silo is when it is wilted but still not dry. In this condition, the leaves show the most effect of the drying, the stems should be green and pliable but not dry and brittle. If the material gets too dry before filling is completed, no hesitancy need be felt about adding water.

Alfalfa may be put into the silo with good results after having been rained on while curing for hay. If the alfalfa was cured, or nearly so, at the time rained on, it may be put into the silo at once after the rain ceases and under these conditions will have about the necessary amount of water to insure preserving qualities.

Fred and Peterson (158) studied the preparation of alfalfa silage according to the method of ensiling hay as reported by Samarani (421). The alfalfa was dried to 30 to 40 per cent water content and tightly packed and heavily weighted in a silo. They reported that the silage produced had a brownish-green color, a pleasant odor, and was readily eaten by cattle. The essentials for successful production of alfalfa silage by this method appear to be the reduction of water to prevent bacterial growth and the retention of sufficient living tissue to remove oxygen. According to Samarani, the principle of the preservation of the hay in the silo by this method depends upon the fact that the plant cells in a few hours transform the oxygen in the silo into carbon dioxide which prevents the growth of bacteria and thus does away with the heating of hay.

Casalis (68) reported good results by adding molasses to alfalfa in making silage. From 0.5 to 0.75 per cent of sugar was added to the material. Sugar is most economically supplied in molasses. Since molasses contains approximately 50 per cent sugar, 30 to 40 pounds of molasses diluted to a working consistency is added to each ton of green alfalfa. Some farmers applied the molasses mixture with watering cans, but others fed it through a blower and cutter. This process is known as the low temperature process and its application produces a fermentation at a temperature not exceeding 30° C. of a preponderantly lactic acid type which will suppress the undesirable microorganisms, such as butyric acid bacteria, responsible for the breaking down and decay of the valuable proteins in the crop. Two milking cows were fed a ration of alfalfa silage and corn silage exclusively for 138 days (95). Alfalfa silage was made with one part beet molasses to each 20 parts of first cutting alfalfa. The two cows showed a preference for corn silage at all times.

Swanson and Tague (460) concluded that silage can be made from sweet clover alone with less difficulty than from alfalfa alone. Dice (102) found that cows on pasture would not eat sweet clover silage made in stacks in any considerable amounts, but when off pasture it was well eaten as a substitute for corn silage. Sweet clover silage was 85 to 90 per cent as efficient for milk production as corn silage in 20-day tests using five cows and five heifers (101). This difference was due to about 10 per cent wastage of the coarser stems of the sweet clover.

Rothwell (416) compared sweet clover silage with corn silage by using 21 cows during a test period of three weeks. The results indicated that 18,220 pounds of sweet clover silage, 206 pounds of grain and 218 pounds of hay were required to replace 18,271 pounds of corn silage.

A test on a large scale was made on a farm near the Missouri Experiment Station in which 70 acres of sweet clover were preserved in silos with excellent results (110). This crop which yielded about 10 tons to the acre was cut when in full bloom. Most of it was allowed to lie in the field until some of the water had evaporated before it was put into the silo. The silage which was well preserved proved to be very palatable for fattening cattle and was fed with entire satisfaction. A sample of typical silage taken from this source contained 35 per cent dry matter. No hesitancy need be felt in preserving sweet clover in the silo if desired. The same precautions should be taken concerning the dry matter content as suggested for other legumes.

If cut while at the first indications of bloom, or before, the green forage should be allowed to lie from three to six hours in the sun to reduce the moisture content. If the crop is more mature no drying is necessary. If through maturity of the plant or over drying, the sweet clover becomes too dry, water may be added freely without any detrimental effects. Bechdel (31) stated in 1923 that roughages composed of clover silage and hay were decidedly better for milk production than hay alone as the sole roughage.

Atkeson and Anderson (20) compared sweet clover silage with corn silage. The sweet clover was cut with a binder and allowed to remain in the field a few hours before being placed in the silo. Eight milking cows were divided into two groups of four cows each. The double reversal system of feeding was used. Each period lasted 20 days. The results indicated that sweet clover silage was practically equal to corn silage for milking cows but that it was not as palatable as corn silage.

Dairy cows when dry can be wintered on legume silage alone (94). Sweet clover cut for silage when it was three and one-half feet high and just coming to bloom contained about 60 per cent water. Corn silage from the previous year was blown into the silo at the rate of one part of corn silage to 20 parts of sweet clover silage. The resulting silage was dark green in color and had a pungent odor which lessened as the silage aged. This silage kept satisfactorily with little spoilage. Four cows, two milking and two dry, were fed this silage. Two cows received silage alone and the other two received in addition three pounds of oat straw daily for a period of 126 days. The cows did not seem to relish the silage, although when forced to eat it, one cow consumed up to 94 pounds daily. Two of the cows consumed an average of 60 pounds per day for 126 days. One cow refused it entirely after 30 days.

The addition of straw to the ration did not affect the amount of silage consumption. The cows lost on an average of 182 pounds in weight. Milk production of both cows dropped greatly.

Soybean silage kept well, had a pleasant odor and cows ate it readily (224). On the other hand Woll and Humphrey (533) found soybeans cut in two-inch lengths when the beans were full sized and firm, but the leaves and stems still green, made a rank, distasteful silage less palatable than corn silage. Three out of 21 cows refused it.

Soybeans containing 30 to 40 per cent dry matter when put in the silo kept well and the loss in feeding value was no more than in case of corn silage (110). The silage required only 40 per cent the storage space required by hay.

Becker and co-workers (38) found that silage of good quality could be produced even when the green soybeans contained as little as 25 per cent dry matter. Putrefactive odors were apparent in the surface spoilage only. Soybean silage was less palatable than corn silage of equal quality. No flavor or odor characteristic of soybean silage could be detected in the milk. More protein and less total digestible nutrients were produced per acre with soybeans than with corn when used as a silage crop.

The addition of one per cent by weight of blackstrap molasses, diluted with an equal weight of water at the cutter, made good soybean silage (121). The soybeans were cut in the early bloom stage with a dry matter content of only 21 per cent. This silage contained 1.22 per cent lactic acid. Large quantities were fed to milking cows with practically no refusal. No objectionable flavors or odors were imparted to the milk of cows receiving soybean silage, even when fed immediately before milking.

Recent Experiments with Grass and Legume Silage.—Although the early work with legume silage indicated the possibility of conserving such a crop in the silo, no great attention was given to this important subject until recent years.

Watson and Ferguson (508) put green grass in a tower silo and compared the resultant silage with artificially dried grass. The silage was palatable and highly nutritious. In later investigations (509) where high protein, artificially dried grass, molasses silage and A. I. V. silage were compared for milk production, all three were highly digestible. There were no significant differences in milk yields, butterfat yields, butterfat percentages, and body weights of the cows due to feeding artificially dried grass, molasses grass silage and A. I. V. grass silage. The carotene content of molasses and A. I. V. grass silage was 49.8 and 57.1 micrograms per gram of dry matter respectively. Both were higher in this respect than artificially dried grass.

Bender and associates (43) reported that low-moisture silage reached a high temperature and much of it spoiled. Chopped silage was superior to long silage in palatability as tested with dairy cows, permitted much more to be stored in the same space and reduced the loss of dry matter (558). Fresh, green grass, either alone or mixed with legumes, when chopped and placed in the silo with no other treatment, made a silage which was highly palatable. The losses of dry matter and carotene were low. Partial drying before chopping facilitated handling, increased the quantity of dry matter that could be stored in a given space, increased the surface spoilage, increased the temperature of fermentation to as much as 120° F., and in most cases made the silage more appetizing. Immature grasses made a more palatable silage than mature grasses. Cows ate good grass silage in about the same quantities that they would graze the grass from which the silage was made.

First cutting alfalfa was dried in the field to a dry matter content ranging from 42 to 73 per cent and successfully preserved in a silo. The total dry matter loss through spoilage on top and through fermentation was estimated to be 7.5 per cent. The carotene content averaged 64 micrograms per gram of dry matter when the crop was put in the silo and 48 micrograms per gram when it was removed. A feeding experiment with milking cows indicated that the alfalfa silage was equal if not superior to U. S. grade No. 2 alfalfa hay (170).

Methods of Filling the Silo.—The method of filling the silo is important (510). Warm fermentation process is achieved by relatively loose packing. Under the best conditions the temperature does not exceed 120° F. Losses, particularly of carbohydrates, must be high. The so-called cold process aims to keep the temperature at or below 80° F., and this end is achieved by cutting the grass in one-half inch lengths in order to facilitate packing, heavy tramping, and the application of pressure to the silo. At Jealott's Hill the best results have been obtained by allowing the mass to rise to a temperature of between 80° and 100° F. The silo is filled in layers, each of which is allowed to heat to this temperature, and finally sealed off with a layer of soil and small movable concrete weights.

Graves (169) reported the results of putting two cuttings of pasture grass in a silo. The first cutting was rather dry and mature and for these reasons water was added at the time of filling. This silage was unpalatable and apparently had undergone undesirable fermentation. The less mature and unwatered second cutting made excellent silage. The following year pasture grass at different stages of maturity were successfully ensiled without any supplement. Pasture grass as well as oats and peas have been preserved successfully in both tower silos and dirt covered stacks (230).

The size of the silo may be an important factor in determining the changes which take place in the silage mass. Experimental work with small containers or with silos with a capacity of 150 tons or more does not give results which can be applied in all cases to the average farm silo of 50 to 100 tons. For instance, there does not appear to be very much difference in the capacity of an average farm silo for corn and alfalfa, while in the case of large silos, the capacity is greater for grasses and legumes than for corn.

The statement is often made that the hay crop can be placed in the silo regardless of weather conditions or the amount of water in the crop. Again this statement may hold for the large silo which is not water proof and where high pressures result in sufficient leakage to reduce the silage to the proper moisture content. If such a silo had no drainage then the quality of the silage made from high moisture material might be low. In case of an average farm silo of 50- to 100-ton capacity the pressure is insufficient to reduce high moisture forage to the desirable moisture content and butyric acid silage may result regardless of drainage facilities.

In filling the silo, the moisture content of the crop, the length of cut and the expulsion of air appear to be important points to consider. The warm process does not appear to be adapted to conditions in the United States since the time required to fill a silo is too great.

Acidity in the Silo.—Allen and Harrison (2) stated that to produce the best type of silage, it is essential that a rapid lactic acid fermentation take place. The acidity thus formed in the mass will prevent the onset of less desirable fermentations such as the butyric acid type. According to Virtanen (485) the development of butyric acid depends on the pH of the mass. Very little of this fatty acid was produced below a pH of 4.2. These results were substantiated by other workers (387, 287, 274).

Watson and Ferguson (510) stated that the ordinary process of silage making did not succeed in controlling the formation of butyric acid, and the amount increased rapidly with the pH, reaching a high level between pH 4.5 and 5.0. The A. I. V. process or the addition of molasses prevented the development of butyric acid. Molasses was somewhat superior to the A. I. V. method in this respect. There was very little butyric acid produced below a pH of 4.2.

The volatile bases produced in silage are also related to the pH. In case of ordinary silage with a pH of 4.91, the volatile bases made up 3.42 per cent of the dry matter, while molasses silage with a pH of 4.19, only 1.67 per cent of the dry matter consisted of volatile bases. A. I. V. silage with a pH of 3.82 had only 1.15 per cent of the dry matter as volatile bases (510).

The relation of the acidity of silage to the production of ammonia has been studied by several investigators (387, 98, 6, 538, 507). It is only when the pH rises above 4.5 that ammonia becomes very high.

The pH value of the silage is the most valuable criterion but it must be reinforced by a determination of the amount of volatile bases and volatile acids (507). When the water content of the silage is 80 per cent, the pH value is high and the butyric acid content is also very high, which is an indication of anaerobic fermentation. The feeding of silage high in butyric acid resulted in ketonuria (51).

Most investigators emphasize the importance of a pH of 4.2 or below for good silage making but excellent alfalfa silage has been produced without any treatment when the pH ranged from 4.61 to 4.78 (170).

Although the buffer capacity of plant material probably plays an important role in making silage from legumes, little attention has been devoted to this subject. In the making of A. I. V. silage allowance is made for the greater buffer capacity of legumes in the application of acids. The neutralizing power of forage crops for organic and mineral acids was investigated by Wilson (526). More acid was required to bring about a change of one pH in leguminous materials than was required by non-leguminous materials. Also, more acid was required to bring about a change of one unit in the region of pH 4.0 than in the region of pH 6.5. The quality of legume silage may be due in part to a variation in the buffer capacity.

Inoculation in Silage Preservation.—The quality of silage depends on the type of fermentation as well as the rate. Green material has been inoculated with various acid-producing organisms. The results of several investigators indicate that lactic acid cultures increased the acidity of the silage, facilitated the fermentation process and improved the quality of the silage (405, 564, 168, 330, 404, 481, 359, 144). Reid (404) using small containers reported that cultures of *Bacillus bulgaricus* organisms were capable of high lactic acid production which kept down the production of acetic and butyric acids. The improved quality of silage resulting from inoculation together with the addition of molasses may have been due to the molasses and not to inoculation. Also, Allen and co-workers (3) found that the losses of starch equivalents in silages made with a solution of dried whey, a solution of dried whey plus culture, and molasses plus culture were 15.3, 17.3, and 3.4 per cent respectively. On the other hand several investigators reported that inoculation did not affect the quality of the silage (159, 507).

The inoculation of ensiled material does not appear to be warranted in view of the limited experimental work which has been reported along this line.

Use of Inorganic Acids.—Various inorganic acids have been added to green plant material in order to stop respiration and control bacterial action. The most widely used is a mixture of hydrochloric and sulphuric acids known as the A. I. V. method which Virtanen (487) has recently described. Inorganic acids in addition to controlling respiration, also control two harmful processes which play a decisive role in the ensiled fresh forage; namely (a) protein breakdown, especially the cleavage of the amino-group from amino acids in the form of ammonia; and (b) fermentation caused by bacteria of the coli-group and by butyric acid bacilli. The reduction of the forage mass below a pH of four prevented the changes which produce abnormal silage. If the silage mass is too high in water, the pH rises during storage to a point where the detrimental breakdown processes immediately set in. Good drainage prevents this decrease in acidity with storage. Virtanen stated that in one experiment, the pH of the artificially acidified silage with good drainage only changed from 3.65 to 3.48 during a storage period of one year. In a corresponding silage, without drainage facilities, the pH rose from its initial value of 3.4 to 5.2.

The A. I. V. method is favored by Virtanen over the sugar or molasses method, due to the fact that considerable loss of nutrients takes place during the first week before sufficient lactic acid is produced to lower the pH below four. The loss of dry matter by this method ranged from 5 to 10 per cent which is very low compared to the losses in haymaking.

Peterson and associates (381) studied the changes in A. I. V. silage made from alfalfa and soybeans. There was no appreciable loss of carotene. The determination of volatile and lactic acid showed that some fermentation took place in spite of the low pH of the silage. No notable changes in the level of milk production resulted from the feeding of the A. I. V. silage. Spectroscopic analyses of the butterfat for carotene and vitamin A were made. During the control period the butterfat contained 4.2 micrograms of carotene per gram and during the A. I. V. period the butterfat contained 6.8 micrograms per gram. Vitamin A rose from 4.0 micrograms per gram of butterfat during the control period to 9.0 in the A. I. V. period. The carotene content of blood plasma paralleled that in the butterfat. The cost of acid per ton of silage ranged from 62.5 to 92.7 cents. Peterson and co-workers also found that alfalfa silage made by the A. I. V. method had considerable more amino, soluble, and ammonia nitrogen.

Various other mixtures of inorganic acids have been used. The Defu process makes use of a mixture of hydrochloric and phosphoric acid. Penthesta is phosphorus pentachloride, a solid which in solution gives phosphoric and hydrochloric acids.

Fagan and Ashton (131) observed that the dry matter losses in grass silage made without treatment, by the A. I. V. method, by the addition of molasses, and by the Defu method were 28.9, 15.5, 9.4 and 9.6 per cent respectively. The loss in haymaking the same year under ideal conditions was 10 per cent of the dry matter in the field and 16 per cent loss during storage or a total loss of 26 per cent. These workers concluded that when the chemical composition, loss of dry matter and palatability of the silage are taken into consideration, the molasses method appeared to be the best suited for preserving grass.

The advantages claimed for the use of phosphoric acid are: (a) helps preserve the forage, (b) increases the phosphorus intake of the animal, and (c) adds phosphorus to the soil (64). A good grade of acid should be used in order to guard against a material high in fluorine. More experiments are needed with this method before it can be evaluated.

Molasses as a Preservative.—Since legumes and some of the grasses are lower in soluble carbohydrates than corn and the cereal grasses (527), molasses or some other source of fermentable carbohydrate is believed to be needed for their successful conservation in the silo.

Several investigators have reported good results with grass and legume silages prepared with molasses (400, 459, 95, 68, 121, 509, 387).

Camburn and associates (64) ensiled immature and unwilted alfalfa with a dry matter content of 24 per cent. This silage was low in acid and exhibited an offensive putrefactive odor. The loss of nutrients was high. The addition of two per cent molasses to this material failed to make a good silage, but four per cent molasses resulted in a fair silage. Alfalfa cut at the same stage, ensiled after being wilted for two hours in the sun to a dry matter content of 34 to 38 per cent made good silage, however, the addition of two to four per cent molasses improved the aroma.

Alfalfa cut in one-half bloom containing 31 to 33 per cent dry matter, when ensiled green without molasses, made a satisfactory silage with a pleasing low acid aroma (64). The addition of two to four per cent molasses made a very good silage. Alfalfa ensiled with 34 to 42 per cent dry matter, without molasses, made an acceptable silage low in acid. An acceptable silage was made from immature timothy before bloom which contained 24 to 27 per cent dry matter. The quality was improved, however, by the addition of one or two per cent molasses. Timothy in full bloom, containing 36 to 39 per cent dry matter when ensiled, resulted in a very satisfactory silage with or without molasses.

Woodward and Shepherd (558) reported the production of excellent silage with alfalfa alone. They emphasized the necessity of fine cutting and an optimum dry matter content. Perkins and associates (376) stated that it has long been known that a good grade of silage can be made from legumes alone. It has been shown repeatedly by experiment that the quality of the silage made by this method is greatly improved by partial drying. The dry matter content must not be so high that the material fails to pack well, because the air must be largely excluded to prevent excessive fermentation and decay. When these requirements were met the Ohio workers were unable to recognize any difference in the quality between silage made without treatment and that made by the addition of various amounts of molasses.

When large amounts of sugar are present in plant materials or added as preservatives to the crops to be ensiled, the fate of the sugars depends upon the conditions in the silo. Wright and Shaw (560) studied the sugar content of corn and sunflowers at the time of ensiling and of the resultant silage. When immature corn containing about 20 per cent total sugar on the dry basis was ensiled, only a trace of the sugar was found in the silage. Corn at the usual silage stage which contained about 12 per cent sugar on the dry basis produced a silage containing more than two per cent total sugars. These results were probably due to a marked difference in moisture content between the immature and mature corn. Fraps reported from 1.87 to 2.39 per cent of total sugars on a dry basis for corn silage (145). In case of legume silage, fermentable carbohydrates would probably not be present except when excessive amounts are added as a preservative.

It is apparent from the literature that good grass and legume silage can be made without the addition of a preservative providing conditions are optimal, dry matter about 30 per cent, and cut in one-fourth to one-half inch lengths and well packed. Most authorities are of the opinion that the use of 40 to 70 pounds of molasses per ton allowed more leeway in putting up grass and legume silage. This may be advantageous under farm conditions. The use of molasses, however, does not insure good silage. Too high moisture content or too much trapped air in silage of low moisture content will result in poor silage regardless of the supplement.

The ensiling of grass and legumes in regions where good haymaking is prohibitive, because of bad weather conditions, appears to be a desirable practice. When such crops are properly ensiled, the loss of nutrients is less than when hay is made. This is especially true of carotene and dry matter. The necessity of using machinery built for such crops should be stressed. The labor required to produce an acre of grass and legumes appears to be less than in the case of corn. Also, the cost of producing 100 pounds of total digestible nutrients in pasture and in various hay crops was less than in the case of cereal grains or corn silage (556). The yield of total digestible nutrients per acre for a 14-year average in Wisconsin was about the same for alfalfa and corn silage (1).

NUTRITIVE VALUE OF THE PROTEIN IN ROUGHAGES

The factors affecting the protein content of roughages have been discussed. The nutritive value of protein depends on the essential amino acid content. Rose (414, 415) has listed the following essential amino acids and their requirement for growth when the non-essential amino acids are included in the food: lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine. Arginine can be partially synthesized by the animal.

Mitchell and Hamilton (339) summarized the results of the early investigators who studied the amino acid content of plant material. Several investigators determined the arginine, histidine, lysine, and cystine content of the protein of alfalfa hay, cocksfoot, corn, spinach, and runner bean (70, 336, 333, 334). The distribution of nitrogen in the various proteins from legume plants is shown in Table 17 (93).

These results indicate that the essential amino acids, such as arginine, histidine, and lysine are present in plant protein in liberal amounts. Tryptophane in the red clover plant varied during growth (485). The tryptophane increased to a high value when the plant was 35 centimeters in

TABLE 17

Nitrogen distribution in leguminous plants

	Alfalfa	Crimson clover	Red clover	Sainfoin	Vetch
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Amid N.	7.70	8.78	6.11	5.55	5.63
Humin N.	6.71	8.38	7.53	6.90	4.95
Arginine N.	18.16	17.29	13.36	12.12	14.88
Histidine N.	7.15	7.87	14.39	12.50	3.96
Lysine N.	5.44	4.49	3.15	9.55	9.56
Cystine N.	1.30	1.17	0.81	0.72	0.84

length. The tryptophane nitrogen made up 2.5 per cent of the total nitrogen but the amount gradually decreased as the plant matured. Virtanen (485) showed that the amino acids of growing plant proteins vary because the growing plant protein has no fixed composition.

Since Rose (414) showed that methionine rather than cystine was an essential sulphur containing amino acid, the amount of methionine in plant proteins is of great importance. Bailey (22) reported that the protein of cocksfoot grass contains 1.7 per cent methionine which accounted for 35 per cent of the total sulphur present.

The total sulphur, inorganic sulphur and organic sulphur of pasture grass cut at different intervals throughout the season showed a decrease with maturity (129). Since 90 per cent of the organic sulphur usually exists as cystine and methionine, it is likely that these amino acids decrease with the maturity of the plants.

Pollard and Chibnall (392) reported that grass proteins contain from 0.3 to 0.95 per cent cystine and that alfalfa leaf protein contained 1.2 per cent cystine. The possibility of a cystine deficiency in roughages was indicated by the work of Haag (189) with rats. Rats fed alfalfa leaf meal as the only source of protein responded to the addition of cystine. Low milk production of cows on a ration of alfalfa alone was believed to have been due to a cystine deficiency (190, 191). Kellermann (265) reported that young rats fed alfalfa as the only source of protein suffered from a deficiency which was immediately relieved by the addition of 0.1 per cent of l-cystine. The nutritive value of alfalfa protein was not enhanced by incubating with "ruminal juice" of sheep. The results of Pollard and Chibnall (392) and Crampton and Finlayson (81) indicate that cystine is not a limiting factor in roughage protein. Lugg (301) reported that plant protein contains 1.1 to 1.7 per cent cystine and 1.2 to 1.6 per cent methionine of the total protein nitrogen. The possibility of an animal deriving cystine from methionine and methionine from cystine was recently reviewed by Toennies (469).

Biological Values of Roughage Protein.—The limited data relative to the amino acid requirement of cattle and the amino acid content of feeds, make it imperative to use biological values as an index of the completeness of proteins for growth and milk production. Nevens (357) obtained a biological value of 62 for alfalfa using rats fed at a 10 per cent protein level. His results also failed to show a supplementary effect of the corn grain proteins with alfalfa proteins. Similar results were secured by Smutts and Malan (442).

Sotola (445) studied the biological value of the protein in the whole hay, stems, and the leaves of first, second and third cuttings of alfalfa hay using lambs. The levels of protein intake for the stems, whole hay, and leaves were approximately 8, 13, and 18.5 per cent respectively. The average biological values of the proteins from the three cuttings were as follows: stems, 64; whole hay, 51; and leaves, 41. The variations in values are attributed to the difference in the levels of protein intake. Sotola (444) also studied the biological values of corn silage and sunflower silage and a combination of alfalfa and corn silage. The resulting biological values were: corn silage, 94; sunflower silage, 67; and a combination of alfalfa and corn silage, 81. These high values were probably due to the low protein content of the rations.

Morris and co-workers (349) investigated the biological value of various proteins for milk production. The biological values for fresh and dried spring grass were 75 to 80, and for fresh and dried autumn grass 60 to 65. These investigators believed that lysine was a limiting factor in milk production. Bartlett (25), however, was unable to substantiate the possibility of lysine being a limiting factor in milk producton of dairy cattle.

Turk and associates (475) working with sheep found that the biological values of proteins for maintenance and growth when fed at a 10 per cent level were as follows: clover protein, 81; alfalfa protein, 78. In an experiment with three lambs, alfalfa hay fed with no additional starch and sugar gave an average value of 50, whereas with starch and sugar added the biological value was 72.

Protein Synthesis in the Rumen.—The hypothesis that bacteria in the rumen synthesize amino acids from non-protein nitrogen was advanced many years ago. Armsby (13) in 1911 reviewed the early work on this problem. The opinion as a result of the earlier work indicated that certain non-protein nitrogen compounds may be used by the ruminant for protein synthesis in the rumen.

More recently Morris (348) and Krebs (285) have reviewed the literature on this subject. Morris pointed out that non-protein nitrogen is often added to a ration already containing more than the requisite amount of protein of good quality.

Woodman and Evans (544) were unable to show that bacteria from the rumen could synthesize cystine. Kotake (282) reported that certain bacteria are able to synthesize tryptophane while others cannot. Ehrenberg and Prittwitz (115) observed a 15 per cent decrease in milk production when 100 grams of ammonium carbonate replaced 500 grams of a mixture of soybean, peanut and cottonseed cake. Similar results were reported by Richter (407) when urea replaced some of the protein concentrate. Richter and Herbst (408) substituted glycocoll or urea for 50 per cent of the crude protein in a basal ration of milking cows. There was a drop in milk production and body weight. The decrease in milk production was less in case of glycocoll.

Recently Hart and associates (194) studied the effect of supplementing a low protein ration containing yellow corn, 30; starch, 20; timothy, 47; steamed bone meal, 2; iodized salt, 1; and cod liver oil, 0.5 per cent with ammonium carbonate, urea and casein. The gain over 14 weeks for the lot of calves on the low protein ration was 65 pounds; low protein ration plus ammonium bicarbonate, 105 pounds; with urea, 110 pounds; and with casein, 126 pounds. The results were explained on the basis of protein synthesis by bacteria in the rumen. Similar results were reported by Bartlett and Cotton (26).

The roughages appear to furnish protein of a high biological value for growth and milk production providing the quantity of protein is sufficient. These high biological values are due to the essential amino acids furnished by these proteins. The possibility of protein synthesis in the rumen is indicated although proof that the microorganisms actually synthesize the essential amino acids is lacking.

The non-protein nitrogen constituents of pasture herbage may bring about abnormal conditions in grazing animals. Greenhill (176) observed that pasture grass which caused serious disturbance among grazing stock was much higher in non-protein nitrogen than pasture grass from pastures where cattle were normal. Seventeen per cent of the nitrogen in normal pastures was non-protein nitrogen, while the grass from pastures in cases where cattle were sick contained 62 per cent of the total nitrogen as non-protein nitrogen.

FAT IN ROUGHAGE AND ITS NUTRITIONAL SIGNIFICANCE

The ether extract of feed is usually considered as being a good index to the fat content. This method of evaluating the true fats of roughages has not been universally accepted. Fraps and Rather (152, 153) and Rather (399) reviewed some of the earlier attempts to separate the fat from the non-fatty substances in the ether extract of plant materials. Rather reported that 68 per cent of the ether extract of roughage was non-fats. In other words, only 32 per cent of the ether extract was true fat. Chibnall and Channon (69) showed that only 27 per cent of the ether extract of leaf protoplasm consists of fatty acids. Recently, Horwitt and associates (240) showed that more than 55 per cent of the ether extract of spinach was non-fat in nature because most of the ether extract consisted of chlorophyll and waxes. Schulze (428) stated that in case of hay and coarse fodders, the nutritive value of the ether extract must be considered low for it consists of wax-like substances and other constituents of doubtful value.

As a class, the roughages are low in ether extract and if only one-fourth to one-half were true fat, then most roughages appear to be very low in this constituent. The low digestibility of the ether extract of hays and fodders is probably due to the presence of waxes, chlorophyll and other substances not easily digested.

Unsaturated Fatty Acids in Roughage.—In view of the work of Burr and Burr (57), who showed that certain unsaturated fatty acids were essential to rats, the fatty acid composition of fats from roughages is of interest.

Smith and Chibnall (438) studied the fatty acid content of cocksfoot and perennial rye grass. The results are shown in Table 18.

TABLE 18

The fatty acid content of roughages

	Cocksfoot		Rye grass	
	Twitchell's method	Bertram's method	Twitchell's method	Bertram's method
	per cent	*per cent*	*per cent*	*per cent*
Saturated acids	11.1	15.2	11.9	16.8
Oleic	12.5	8.9	22.5	17.6
Linoleic	33.4	29.8	26.1	21.2
Linolenic	25.8	29.5	39.5	44.4

Speer and associates (447) working with dried spinach which contained 0.8 per cent true fat reported that about five per cent of the true fat was solid and that 26 per cent was liquid. The liquid fat was made up, in part, of 12.7, 34.7, and 26.3 per cent of linolenic, linoleic and oleic acids respectively. The solid fat consisted chiefly of palmitic, stearic with about three per cent cerotic acid.

It is interesting to point out that the true fats in the limited number of roughages studied were rich in the unsaturated fatty acids, which probably precludes a fatty acid deficiency among cattle fed liberally on pasture and hay providing the true fat of roughages commonly used have a similar composition.

Necessity of Fat in the Ration for Milk and Fat Secretion.—Recent studies by Maynard and associates (311, 312) and Gibson and Huffman (163) have suggested that a certain minimum level of fat is needed in the ration of the lactating cow for optimum milk production. The work of Graves and associates (173) with alfalfa hay alone indicated that fat per se may not be a limiting factor in milk production on roughage alone. Recent work lends further support to this idea (245).

The effect of food fat on the butterfat percentage of milk has also been investigated. Most of these studies indicated that food fat did not influence the percentage of fat in milk. Recently, however, several investigators have demonstrated that cod liver oil feeding decreased the test (378, 166) while other oils increased the test of the milk (4, 161, 163) for a brief period. Powell (394) concluded from his experiments that the per cent of fat in the milk may be lowered considerably by changing from whole hay to hay and grain in pellet form. The feeding of alfalfa alone resulted in tests slightly higher than normal according to Graves and associates (173).

The trend toward greater roughage utilization and the use of low fat concentrates suggests a need for more information regarding the role of fat in milk and fat production.

CARBOHYDRATES IN ROUGHAGES AND THEIR NUTRITIVE VALUE

The carbohydrate fraction of feeding stuffs contains a wide variety of substances; the structural parts such as cellulose, lignin and pectin, and other constituents such as starch, fructosans, pentosans, and various sugars. Since the carbohydrate fraction represents about 75 per cent of the dry matter of the plant, their importance in the nutrition of cattle is apparent.

In the young growing plant, the tissues consist of cells largely filled with protein substances and cell walls made up of protein and cellulose. The adjacent cells are cemented together with pectins and the so-called hemicellulose material. As the cells mature, the protein of the cell walls disappear and is replaced by a deposit of hemicellulose and later by lignin.

Strepkov (453) has recently separated the carbohydrate complex into seven groups as follows:

1. Hot alcohol-soluble (sugar).
2. Cold water-soluble, but alcohol insoluble (dextrins, gums, pectins, B-amylan).
3. Soluble in water at 45°–47° C. (inulin).
4. Hydrolized by diastase (starch and glycogen).
5. Soluble in hot water (protopectin, zylan and araban).
6. Hydrolyzed by 2.5 per cent H_2SO_4 (galacten, mannan, pentosan).
7. Not hydrolyzed by dilute H_2SO_4 (lignin, cellulose and manno-cellulose).

Nitrogen-Free Extractives of Roughages.—The nitrogen-free extractives of feeds are estimated by subtracting the sum of the percentages of moisture, ash, protein, ether extract and crude fiber from 100. The constituents of this fraction of roughages have received little attention from the standpoint of chemical analyses and nutritive value. Some of the early investigations were made by Frear on timothy hay (157) and by Headden (211) on various Colorado feeds. Fraps (145) in 1900 reported the sugar content of the various feeding stuffs as indicated in Table 19.

The starch content of roughages was also studied (147). Alfalfa hay, corn silage, prairie hay, and Sudan grass hay contained 1.9, 6.0, 2.1 and 2.2 per cent starch respectively on a dry basis.

TABLE 19

The sugar content of various feeding stuffs

	Reducing sugars	Sucrose	N. F. E.	Total sugars	Total pentosans
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Alfafa hay	3.00	1.40	41.1	4.40	.....
Mixed hay	8.00	2.44	48.7	10.44	. .
Timothy	4.97	3.36	55.9	8.33	24.9
Crab grass	1.42	0.47	45.2	1.89	26.2
Green rape	4.14	2.97	44.4	7.72	9.6
Corn silage	2.01	0.11	53.0	2.13	.....

Guanzon and Sandstrom (181) recently studied the composition of the residual nitrogen-free extract fraction in nine feeding stuffs. Alfalfa hay with 40.4 per cent nitrogen-free extract contained 1.76 per cent reducing sugars, 1.65 per cent sucrose and 1.4 per cent starch.

The soluble carbohydrates in forage plants was studied by Wilson and Webb (527). Their data indicate that most of the legumes contain from four to six per cent soluble carbohydrates on the dry basis, while timothy, perennial rye grass and brome grass contain about eight per cent. The cereal grasses, barley, oats, rye, and wheat contain from 10 to 13 per cent and corn in milk about 20 per cent soluble carbohydrates.

In the corn plant sucrose is the characteristic carbohydrate of the vegetative portion (300). Most of this is stored in the stalk. The curing of corn in the field results in the loss of sugars, about the same loss that occurs in the silo. In the silo, however, the lactic acid formed from the sugar contributes to the nutrition of the animal.

The residual nitrogen-free extract of roughages may contain chlorophyll, organic acids and ligno-cellulose (147). Feeds known to be high in feeding value are characterized by a high content of sugars and starches, or if the starch content is not high, by a high protein content. The residual nitrogen-free extract is low in such feeds. On the other hand feeds which are known to be low in feeding value are generally characterized by high pentosans and residual nitrogen-free extract contents. Pentosans are usually low in concentrates and high in roughages while the reverse is true for starch.

According to Norman and Richardson (369) young rye grass is charac-

TABLE 20

Composition of nitrogen-free extract of feeds (181)

	Uronic acid anhydrides	Sugars and starch	Pentosans total	Residual nitrogen-free extract
	per cent	*per cent*	*per cent*	*per cent*
Alfalfa hay	25.76	12.01	25.51	62.50
Corn cobs	9.99	6.91	52.74	40.33
Timothy hay	10.60	12.08	28.90	58.99

terized by a high percentage of water-soluble material, only a small amount of which is nitrogenous. The major part was fructosan which decreased sharply as the grass matured. The free sugar consisted largely of fructose, although no particular significance has been attached to this finding because free fructose may be a result of the drying procedure. The dry matter of the young grass consisted of about 25 to 30 per cent fructose after hydrolysis while the hay varied from 14.6 per cent for that cut in June to 0.4 per cent for that cut in September. The leaves, stems and heads of the grass cut on June 2 contained 24.4, 35.4 and 17.3 per cent respectively of fructose after hydrolysis. Most of the fructose after hydrolysis was derived from fructosan since the grass contained only 1.64 per cent total reducing sugar expressed as fructose, of which 1.57 per cent was actually fructose.

The fructose, soluble protein and soluble ash constituents together did not account for all the cold water-soluble material. The presence of a glucose polysaccharide has been postulated. Cellulose, which continued to increase after the dry weight of the plant reached its maximum, may increase at the expense of stored fructosan.

Second growth rye grass was characterized by higher structural elements and lower amount of water-soluble constituents, such as fructosan, when compared with first cutting grass. This may account for the belief that autumn grass is not as satisfactory as spring grass for livestock even though the protein may be the same. Norman (368) stated that it is probable that the fructosans are of great nutritional importance because of their high availability.

A study of the carbohydrates in the wheat plant revealed a maximum sucrose content in the leaf on March 16; in the leaf sheath on June 8, and in the stem and head on July 5. Sugars fell rapidly with starch formation in the grain. Fructosans were present and were especially high in the young heads but fell rapidly as ear development and starch formation proceeded (23).

According to Norris and Resch (370) pectin, which is a constituent of young plant cells, is a complex mixture of substances having similar properties. It is probable that the usual sample of pectin consists of a central nucleus of galacturonic acid units to which arabinose and galactose units may be chemically attached in chains.

In a study of the nature and distribution of cell wall constituents, Buston (58) reported that fully grown cocksfoot grass contained 1.97 per cent pectin as calcium pectate. Hay made from the grass contained 1.95 per cent calcium pectate. The pectic substance in bean leaves varied from 6.5 to 8.9 per cent of the dry matter as calcium pectate. Norman found (367) that young rye grass contained 0.91 per cent pectate. The pectin content decreased with the maturity of the rye grass and when cut for hay it contained only 0.19 per cent. According to Norman, pectin is a cell wall constituent of the young plant cell which is replaced by cellulose and lignin at maturity.

The role of pectic substance in nutrition has been investigated by several workers. Recently Sullivan and Manville (455) showed that pectin and cellulose together with vitamin A are essential in the digestive tract for the synthesis of the enzyme lysozyme which aids in the control of the bacterial flora of the intestine.

The nutritive value of pectins in nutrition has received scant attention. Certainly more research should be devoted to this material in view of the possibility that the pectic substance along with easily fermentable cellulose may contribute to the succulence of young pasture grass.

Fraps (146, 147) also studied the coefficient of digestibility of the constituents of the nitrogen-free extract using sheep. The results for a few roughages are shown in Table 21.

TABLE 21

Coefficient of digestibility of constituents of N. F. E.

	Reducing sugars	Polysaccharoses	Starch	Pentosans in N.F.E.	Residual N. F. E.	Total pentosans	Pentosans in C.F.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Alfalfa hay	97	98	86	56	69	53	41
Bermuda hay	97	99	90	44	48	44	43
Corn cobs	84	79	83	53	34	55	65
Corn silage (dry)	99	94	97	77	68	56	72
Kafir forage	94	97	95	43	42	54	46
Milo forage	81	79	60	57	41	62	68
Prairie hay	97	94	77	52	35	50	43
Sorghum hay	99	93	83	49	48	65	55
Sudan grass	99	84	77	53	38	55	58

The reducing sugars and sucrose of roughages were almost completely digested. The total pentosans in hays and fodders are generally digested to the extent of about 50 to 60 per cent. The digestibility of the pentosans of timothy hay cut in the early bloom stage ranged from 55 to 60 per cent. According to Fraps a pound of digestible nitrogen-free extract in hays and fodders is much less valuable to the animal than a pound digested from concentrates.

The soluble constituents of the nitrogen-free extract fraction of roughages are probably the most important parts from the standpoint of productive energy value. The losses due to respiration, fermentation and leaching of roughages may account for the marked variation observed in the nutritive value of roughages. The dissipation of the soluble carbohydrates of roughages may also account for the susceptibility of cattle to ketosis. This phase of the roughage question will be discussed in connection with the rumen digestion of cellulose.

Nutritive Value of Crude Fiber.—According to Armsby (14) crude fiber contains most of the cellulose, lignin and cutin, along with some of the more difficultly soluble hemicelluloses, particularly those containing pentosans.

Headden (210) studied the chemical composition of alfalfa in early bloom stage. He found that the crude fiber contained 79.0 per cent cellulose and 21.0 per cent lignin. The complete chemical composition of alfalfa-clover hay was given.

Norman (366) concluded that the crude fiber determination is misleading because the fraction obtained does not bear any definite relationship to the structural constituents of the material. The cellulose is partially attacked and the lignin is extensively removed. Highly lignified materials do not necessarily yield a crude fiber high in lignin. Crampton and Maynard (82) separated the crude fiber fraction into cellulose and lignin. They also concluded that the separation of crude fiber into its various components was highly significant from a nutritional point of view.

As has previously been stated, the cellulose and lignin content of plants increases with the rate of maturity. Williams and Olmsted (524) reported that alfalfa leaf meal contained 32.5 per cent cellulose and 1.5 per cent lignin. Norman (366) studied the composition of the crude fiber fraction in several feeds. The results are shown in Table 22.

TABLE 22

The composition of crude fiber on the basis of dry matter

Material	Crude fiber	Composition of crude fiber	
		Cellulose	Lignin
	per cent	*per cent*	*per cent*
Bean straw	45.8	76.8	18.6
Oat straw	35.2	92.0	6.8
Wheat plants	32.6	92.4	6.4
Hay	31.5	85.7	10.9
Vetches	31.1	74.3	20.2
Corn plants	22.6	96.5	3.5
Kale plants	17.3	76.2	18.1
Sugar beet pulp	14.5	87.9	10.6
Bran	11.3	93.0	2.1

About 97 per cent of the crude fiber fraction was made up of cellulose and lignin.

Williams and Olmsted (524) determined the cellulose and lignin content of alfalfa leaf meal and cabbage. Both were high in cellulose on a dry basis but alfalfa leaf meal contained 15.0 per cent lignin while cabbage contained only 2.6 per cent. Beet pulp and carrots were very low in lignin.

Kellner (268) in 1905 showed that if the incrusts in straw (silica and lignocellulose) were removed by treatment with alkaline reagents then the digestibility of the cellulose in the residual product was extremely high.

Ustyantzev (479) in 1906 observed that wheat straw, when fed to sheep, had a low nutritive value but that cellulose from the same straw freed from the incrusting substance was equal to isodynamic quantities of starch and sugar as protectors of protein and fat. Fingerling and associates (135) reported that pigs were able to dissolve pure crude fiber as are ruminants but that they lose this ability as the incrustation or lignification of the fiber increases.

Woodman (536) demonstrated the relative digestibility of the fiber in young and mature plants as shown in Table 23.

TABLE 23

Digestion coefficients of constituents of grass in its young leafy stage of growth and at the hay stage of maturity

	Young leafy grass	Grass at hay stage of maturity
	per cent	*per cent*
Fiber	84.2	52.4
Carbohydrates	97.4	53.0
Protein	85.4	50.0
Fat	60.0	30.0

The digestibility of fiber, therefore, appears to be inversely proportional to its content of lignocellulose. Woodman also showed the difference in the digestibility of non-lignified and lignified feeding stuffs. Non-lignified feeds such as sugar beet pulp, mangolds, cabbage and sugar beet tops showed coefficients of digestibility of the cellulose as 89.7, 78.0, 74.0, and 71.6 per cent respectively. On the other hand, the coefficients of digestibility of the cellulose of such lignified feeds as oat straw, wheat straw, cottonseed meal, linseed meal and wheat bran were only 54.0, 50.0, 37.0, 32.0 and 26.0 per cent respectively.

Lignin occurs in mature plants chiefly as lignocellulose. As already indicated, lignin is believed to be responsible for the marked drop in digestibility of forage during development. Crampton (79, 80) using rabbits studied the effect of lignin on digestibility of cellulose. He observed that as the lignin increased the coefficients of digestibility of the dry matter decreased. These data support the work of Prjanischnikow and Tomme (397) in which they observed that after the treatment of rye straw with ClO_2, the digestibility of its fiber by rabbits increased from 15 to 79 per cent. This was probably due to the partial removal of the lignin.

The digestibility of lignin has been reviewed by Crampton and Maynard (82). The results from the available literature are conflicting. These workers studied the digestibility of lignin by rabbits and a steer. In the case of rabbits, 97.8 per cent of the food lignin was recovered in the feces, while 99.3 per cent was recovered in the case of the steer. It appears that lignin is almost biologically inert.

Crampton and Maynard (82) proposed a scheme of carbohydrate partition in feed analysis into lignin, cellulose, and other carbohydrates, which may have more biological significance and hence be of greater usefulness in predicting feeding values than the present division into crude fiber and nitrogen-free extract. In the case of the steer the coefficient of digestibility of the nitrogen-free extract by the standard method was 67 while by the suggested scheme it was 92 per cent.

The separation of the crude fiber fraction into cellulose and lignin appears to be a step in the right direction. The old crude fiber method did not differentiate between non-lignified and lignified material and consequently failed to predict the nutritive value of the roughage.

Rumen Digestion.—The rumen digestion of roughage high in fiber has been studied by many investigators. Much of the earlier work was reviewed by Woodman (536). Recently Pochon (389, 390, 391) concluded that fatty acids were the principal end-products of rumen digestion and not glucose as had been postulated by Woodman (536). Woodman and Evans (546) has recently reported results which substantiate Pochon's findings. Pochon isolated an organism (*Pletridium cellulolyticum*) which is capable of digesting cellulose. This facultative anaerobe was isolated from the rumen of cattle and sheep. In the fermentation of filter paper with filtered rumen contents and $CaCO_3$ in an alkaline medium, the end products were formic acid, acetic acid and some propionic acid and ethyl alcohol but no reducing sugar. When the reaction of the medium was adjusted to a pH of 4.0–5.0, or when toluene was added, the cellulolytic activity was markedly reduced and glucose was the main end-product.

The pH of the saliva is 8.4 and aids in the neutralization of the end-products of fermentation (271). Monroe and Perkins (344) studied the pH of rumen content of cows fed on various rations eight hours after feeding. The pH was 7.0 when grain and hay were fed and the feeding of corn silage or A. I. V. silage did not change the pH of the rumen appreciably.

The importance of rumen digestion is not commonly appreciated. A large number of roughages consist of constituents which are not digested by enzymes elaborated by the animal organism but are broken down by microorganisms in the rumen. Approximately 50 per cent of alfalfa is dependent upon rumen fermentation for its digestion. These constituents include cellulose, compound celluloses, pentosans, levulosans, galactans, mannans and pectic substances.

The observation made by early workers that a pound of digestible cellulose was equal in nutritive value to a pound of digestible starch points to the production of end-products of rumen fermentation which can be converted to net energy in the animal body. The *in vitro* experiments of Pochon, where a preponderance of the end-products were acetic acid, do not solve the problem since acetic acid is not regarded as a source of chemical energy but is changed to thermic energy, which would be of little value under most conditions of feeding.

Possible Relation of the Rumen to Ketosis.—Ketosis is a disease characterized by the inability of animals to completely burn fat. The incompletely burned fatty fragments are called ketone bodies. Their accumulation in the body of cattle result in a drop in milk production, in a decrease in appetite, and in body weight in the more pronounced cases. A nervous type of ketosis has been observed occasionally. Ordinarily this condition is due to a lack of available soluble carbohydrates in the small intestine. Ruminants are more likely to develop ketosis than other classes of livestock. This may be due to the conversion of foods which stagnate in the rumen for an appreciable length of time, to fatty acids by the rumen flora. The soluble material has a greater chance of getting past the rumen without being acted on by bacteria and consequently is effective in the prevention and cure of ketosis. A certain type of rumen flora produced ketone bodies (279). Cattle are more likely to suffer from ketosis during the winter in sections where the roughage is low in soluble carbohydrates. As previously indicated, hay may lose soluble carbohydrates due to respiration, fermentation and leaching. The conservation of soluble carbohydrates in roughages for winter feeding is an important health and economic problem in dairy cattle nutrition.

ROUGHAGES AND MINERALS

Calcium.—As a general rule, roughage is a good source of calcium and a poor source of phosphorus as shown by the average analyses of feeds (350, 340).

In the early experiments dealing with the effects of low calcium rations, the roughages were probably low in carotene, consequently, reproductive failures and other symptoms which were attributed to a lack of calcium were due in fact to a carotene deficiency (199, 329). Early metabolism trials also indicated a great loss of calcium from the body of milking cows regardless of the calcium intake (139, 198, 323, 327). More recently, short-time balance trials in connection with long-time feeding experiments indicate that cattle utilize about 50 per cent of the calcium intake on low calcium rations (249, 87). Long-time metabolism trials (117, 118) also gave similar results. Cows on low calcium rations for a long time showed no ill effects (328, 373).

Becker and associates (39) observed broken hips and bones of low breaking strength among cows fed limited roughage. The calcium requirements for maintenance and milk production have not been definitely worked out. Mitchell (340) suggested 26.4 grams of calcium daily for a 1,200 pound cow producing 30 pounds of milk testing 3.5 per cent fat.

It is apparent from the literature that the liberal feeding of roughage containing about 0.30 per cent calcium on the dry basis furnishes an ample supply of this element for maintenance and liberal milk production.

The calcium requirement for growing calves appears to be very low when they are supplied with ample vitamin D. Theiler and co-workers (465) obtained fair growth in heifers fed a ration containing five grams of calcium daily. Good growth and normal bone formation occurred in dairy heifers fed a ration containing 6 to 21 grams of calcium daily from birth to two years of age (243). The low calcium ration used by Lindsey and associates (298) contained too much calcium for critical interpretation. The rickets-like condition of young cattle observed in the field by Gullickson and co-workers (187) was associated with very heavy grain feeding and very light roughage feeding. The condition was attributed to an insufficient supply of vitamin D and calcium. Under normal conditions of liberal roughage feeding this condition is not observed.

The greater use of good roughages, especially legumes or mixtures containing legumes, assures an adequate supply of calcium for growth, reproduction and milk production.

Phosphorus.—Phosphorus deficiency occurs among cattle, especially milking cattle, in many localities throughout the world. This is shown in Table 24.

TABLE 24

Phosphorus and calcium content of roughages reported from various localities

Author	Geographic location	Crop	Insufficient		Sufficient	
			P	Ca	P	Ca
			per cent	*per cent*	*per cent*	*per cent*
Orr (372)	Scotland	Pasture	0.137	0.150	....	....
Stohmann, cited by Eckles et al (111)	Germany	Hay	0.092	0.450	0.188	0.645
Kellner, cited by Eckles et al (111)	Germany	Hay	0.113	0.593	0.188	0.678
Guthrie, cited by Eckles et al (111)	Australia	Hay	0.121	0.270	0.121	0.393
Henry, cited by Orr (372)	Australia	Pasture	0.121	0.273	0.245	0.296
Tuff (474)	Norway	Hay	0.070	0.257	0.191	0.628
Dircks, cited by Tuff (474)	Norway	Marsh hay	0.050	0.132	....	....
Aston (19)	New Zealand	Pasture	0.118	....	....	....
			0.083	....	....	....
			0.114	....	....	....
Theiler et al (464)	South Africa	Pasture	0.107	....	0.140–0.262	....
Hart, et al (193)	Wisconsin	Sweet clover	0.098	....	....	....
			0.143	....	....	....
Scott (429)	Montana	Alfalfa	0.124	1.749	0.173	1.485
		Clover	0.131	1.642	0.147	1.142
		Wild grass	0.074	0.320	....	....
		Red top	....	....	0.146	0.264
		Bunch grass hay	....	....	0.142	0.221
Eckles, et al (113)	Minnesota phosphate deficiency area	Alfalfa	0.212	....	....	....
		Timothy	0.106	....	....	....
		Prairie hay	0.112	....	....	....
Eckles (111)	Minnesota	Alfalfa	0.190	....	0.257	....
		Timothy	0.107	....	0.135	....
		Prairie hay	0.100	....	0.108	....

The symptoms of phosphorus deficiency in cattle were described by Theiler (464) as osteophagia (bone chewing), pica (craving for things not ordinarily classed as food), stiffness and anorexia. Anorexia is the principal deficiency symptom of severe phosphorus deficiency (248, 276). Under such conditions, bone and wood chewing do not occur until partial recovery has taken place. Pica or depraved appetite is not usually manifested by cattle when the blood plasma inorganic phosphorus is about one mgm. per cent.

Several investigators observed that a lack of phosphorus in the ration resulted in a decreased food utilization (464, 112, 111, 113, 409, 276). Forbes (138) failed to show a difference in growth or in the utilization of food energy or protein between rats fed low phosphorus and high phosphorus diets. The efficiency of food utilization in uncomplicated phosphorus deficiency in dairy cattle is difficult to measure, due to the development of anorexia.

The phosphorus requirement of dairy heifers from birth to first calving is about 6 to 12 grams per day (248). Archibald and Bennett (9) stated that heifers can make average growth on rations supplying 1.8 grams of phosphorus per 100 pounds of live weight during the first year of life, 1.7 grams during the second year, and 1.2 grams during the third year.

Uncomplicated reproductive failures were not attributed to a phosphorus deficiency but the disturbance in estrum and the low calf crop among cattle in phosphorus deficient areas, under natural conditions, was probably due to complicated nutritive deficiencies prevalent under such conditions (114).

The phosphorus requirement for lactation was determined by Huffman and associates (248, 289) from balance trials and a study of the relation of phosphorus' intake to blood inorganic phosphorus. Milking cows require about 0.7 gram of phosphorus per pound of milk above maintenance when 10 grams of phosphorus is allowed per 1,000 pounds of live weight. Mitchell (340) suggested a lower requirement which may be satisfactory when roughages other than alfalfa are used or it may apply to cows which have had a high phosphorus ration for years. In the experiment reported by Huffman and associates, the cows were raised on a low phosphorus ration from early age.

Calcium-Phosphorus Ratio.—The Ca/P ratio apparently has very little effect on the efficiency of calcium and phosphorus utilization in case of dairy cattle. Ratios from 1:4 to 10:1 have not interfered with calcium and phosphorus utilization (243) (191). An extreme ratio of about 20:1 appeared to depress phosphorus utilization (113). Theiler and associates (463) stated that the decreased food consumption of phosphorus deficient animals was not wholly due to inadequacy of the phosphorus but was also associated with the calcium content of the ration. Marek and associates (305), concluded that osteomalacia in cattle may be due to the feeding of rations too high in calcium and magnesium.

The trend toward greater utilization of roughages through pasture, silage and hay tends to accentuate a phosphorus deficiency. As a rule, roughages are low in phosphorus and grains are medium to high in this element. When appetizing roughages which are high in protein are fed, high producing cows may not receive sufficient phosphorus. When roughage containing 0.2 per cent phosphorus or less is fed a phosphorus supplement low in fluorine should be supplied in order to insure a good appetite for roughage. Usually a mixture of odorless steamed bone meal and salt equal parts, fed ad. lib. is a satisfactory supplement.

Iodine.—The iodine content of roughages depends on the iodine content of the soil (320). The iodine content of corn grown on soil to which increasing amounts of potassium iodide were added during the growing season showed a marked increase. The iodine content of roughages varies markedly as indicated in Table 25.

In sections where the soil is low in iodine the plants may be low in this element. The data included in Table 25 for spinach from various states are given to illustrate this point.

The water supply also contributes iodine to the animal. When the foods and water are low in this element "big neck" or goiter occurs among new-born calves. The iodine requirement for reproduction is 400 to 800 micrograms per 1,000 pounds of live weight (340). On farms where goiter occurs among new-born calves iodine should be added to the ration preferably as iodized salt. There is no indication that an iodine supplement is advantageous under other conditions. Regarding the relative merits of organic and inorganic iodine, the statement that "iodine is iodine" seems approprate.

Manganese.—The manganese requirement of cattle is not known. The amount of this element in roughages depends on the reaction of the soil as well as the amount present (423). As a general rule, the grasses are relatively good sources of manganese. Alfalfa was found to vary from 11 to 63 milligrams per kilogram but the stage of maturity was not a factor. Oat hay was found to vary from 25 to 280 milligrams per kilogram depending on the acidity of the soil. Roughages grown on acid soil were higher in manganese than those grown on alkaline soil. Other roughage varied in manganese in a similar manner.

Recently Blakemore and associates (48) attributed grass tetany in cattle to an excess of manganese in the pasture grass. Pastures where this disease occurred were much higher in this element than were healthy pastures. The feeding of manganese salts at rather high levels depressed blood magnesium in animals. This work has not been confirmed by other workers.

Iron, Copper and Cobalt.—Nutritional anemia among cattle and sheep has been observed in certain widely scattered areas of the world. This condition has been called "bush sickness," "skinnies," or "Morton Mains ailment" in New Zealand; "daising," "pining" or "vanquish" in Scotland;

"lecksucht" in Holland; "Nakuruitis" in Kenya Colony, East Africa; "coast disease" in King Island, Tasmania; "salt sick" in Florida and "neck ail" in Massachusetts. The literature on this problem has recently been reviewed by Neal and Ahmann (355) and Marston and associates (308).

TABLE 25

Iodine content of roughages

Feed	Micrograms per Kg.	Location
Pasture grass (165)	260–2020	Scotland—various parts
" "	2.3–170	England— " "
Alfalfa (424)*	560–5750	Germany
Red clover*	630–2000	"
Oat straw*	360–2050	"
Barley straw	950–28000	"
Grass (165)	153–5000	?
Hay	56–440	?
Grass hay (341)	34–448	South Carolina
Clover (crimson)	33–89	" "
red	218	" "
alsike	170	" "
white	33	" "
Alfalfa		
Peruvian	352	" "
Kansas	86	" "
Dakota	72	" "
Vetch		
Purple	504	" "
Monantha	302	" "
Hairy	504	" "
Woolsey pod	504	" "
Soybean	trace to 403	
Pasture grass		
Bermuda grass	100	" "
Lespedeza	150	" "
Johnson grass	350	" "
Pasture** (77)	190–450	Somerset, England
" ** (77)	130–500	Suffolk, England
Clover	190	Jealott's Hill, England
Cocksfoot	190	" " "
Yorkshire	390	" " "
Meadow Foxtail	270	" " "
Spinach (217)	694 (dry basis)	South Carolina
"	567 " "	Georgia
"	32 " "	California
"	19.5 " "	Oregon
"	226–1083	Oklahoma

* fertilized.
** survey.

The results of early investigations indicated an iron deficiency but iron supplements in certain localities failed to produce uniform results. In 1935, Marston (307), Underwood and Filmer (477), Grimmett and Shoreland (178) and Lines (299) demonstrated the value of cobalt as a supplement to pastures where this type of nutritional anemia occurred.

Hopkirk and Grimmett (238) reported that healthy pastures usually contained more than 0.07 parts per million of cobalt. McNaught (321) found that "bush-sick" pastures averaged less than 0.04 parts per million of cobalt while pastures from healthy areas averaged 0.11 parts per million but varied with the season. Askew and Maunsell (18) and Askew and Dixon (17) increased the cobalt content of herbage by the application of cobalt chloride.

Becker and Gaddum (37) and Neal and Ahmann (355) found that the use of iron and copper supplements aggravated the disease which was prevented or cured by cobalt. Workers in Australia (308) have reported that the treatment of "coast disease" with cobalt alone permitted growth in some animals but failed in most cases to prevent the progressive development of anemia. Treatment with copper and cobalt prevented the development of anemia. The administration of cobalt chloride and nickel was more effective than cobalt alone in the treatment of Morton Mains ailment (105).

Table 26 gives the copper content of forage crops reported by different investigators.

TABLE 26

Copper content of roughages on the dry matter basis

Forage	Copper	Forage	Copper
	mg. per Kg.		*mg. per Kg.*
Bluegrass (316)	7.5	Alfalfa hay (319)	10.0
Kentucky bluegrass (317)	7.5	Alsike clover	6.9
Alfalfa foliage	8.0	Bluegrass	14.0
Red clover foliage	8.0	Orchard grass	5.0
Kentucky bluegrass (318)	14.0	Red clover hay	17.0
Alfalfa hay (124)	9.1	Red top	4.0
1st cut. (Wis.)	4.5	Soybean	8.0
2nd cut. (Wis.)	14.8	Sweet clover	9.0
Colo. sample 1	6.9	Timothy hay	5.0
Colo. sample 2	11.9	Wheat straw	3.0
Bluegrass	8.3	Lecksuch area hay av. (437)	2.0–3.0
Oat straw	3.8	" one case	3.0–5.0
Red clover	17.6	" one case	1.0
Rye straw	4.4	Normal hay av.	6.0–12.0
Soybean hay	8.2	" " one case	5.0
Sweet clover hay	11.8	Normal grass or pasture	20.0–30.0
Timothy hay	2.2		
Vetch hay	9.6		
Wheat straw	2.1		

Archibald and associates (10) reported that nutritional anemia, called "neck ail" by the farmers, occurred in several localities in Massachusetts. These investigators concluded that the anemia was due to an insufficient amount of iron in the native forage. Spectacular recovery followed the administration of iron compounds to the affected animals. The addition of iron compounds to the soil from farms where the disease had occurred, resulted in a uniformly large increase in the percentage of iron in grasses grown on these soils.

A deficiency of iron, copper or cobalt or a combination of these elements is very unlikely to occur except in isolated areas. Most roughages are good sources of these blood-forming elements. Certainly, the general use of commercial supplements furnishing these elements is not warranted.

Other Minerals.—Magnesium is found abundantly in most roughages. Since the requirement for growth is only 8 to 10 milligrams of magnesium per pound of body weight, the possibility of a magnesium deficiency in the ration is indeed remote (247). The disturbed magnesium metabolism which occurs in grass tetany and milk fever is probably not associated with the magnesium content of the rations.

There is also very little likelihood of a potassium deficiency among cattle since this element is found in large quantities in most feeds, especially roughages. Lincoln (296), however, after analyzing 60 samples of pasture herbage in Mauritius, concluded that the pastures were deficient in nitrogen and potassium and that the phosphorus content was usually low.

Many feeds are low in sodium and chlorine because of the low sodium and chlorine content of the soil in many localities. Common salt supplies these elements. "Alkali disease" or selenium poisoning among cattle is due to the toxic effect of selenium in feeds (353). Fortunately the areas affected with this disease are not wide-spread. Ferguson and co-workers (134) reported that a large area of pasture in Somerset could not be grazed by cattle due to the toxic effects of molybdenum in the grass.

ROUGHAGE AND VITAMINS

Roughages as a Source of Carotene.—The plant synthesizes carotene, consequently, roughages as a carrier of this food constituent are of great importance. Virtanen (486) found that carotene was higher in the rapidly growing plant and reached a maximum value before or at the beginnig of flowering. He also observed that proper fertilization increased the carotene content while factors which retarded growth lowered the carotene content. The vitamin A content of young alfalfa (10–12 inches high) was much greater than that of alfalfa in the bloom stage (205). The vitamin potency of alfalfa is located chiefly in the leaves.

The carotene content of rye, Kentucky blue grass, red top, Dakota brome, orchard grass, big blue stem, Canadian brome, little blue stem and local brome grass was determined throughout the pasture season (21). All pasture grasses studied showed a relatively high carotene value in early summer, but with wide variations. The carotene content decreased markedly during the hot months of midsummer. After the fall rains, the carotene content increased with the exception of the big blue stem and Buffalo grass.

The vitamin A of grama range grass when cut in August showed marked potency. Approximately twice as much of the grass cut in September and 100 times as much cut in November was necessary to produce the same rate of gain in rats (441). Hay cut at the early bloom stage contained more vitamin A, as a rule, than that cut at other stages of growth, the method of curing being the same (107).

Losses of Carotene during Roughage Conservation.—Although grasses and legumes are high in carotene when cut at the proper stage of development, the resulting field-cured hay is extremely variable in this respect. Russell (417) observed that leaves from plants dried by artificial heat contained seven times as much vitamin A as leaves from hay that were cured in the field. Hay exposed to the sun without exposure to dew or rain showed a loss of vitamin A.

The exposure of alfalfa hay to sun, rain or dew over a period of several days resulted in a marked loss of vitamin A (45). There was a 75 per cent loss of vitamin A when alfalfa was left in the field over night (440). Alfalfa bleached severely as a result of one week's exposure to sun and rain in the swath contained but four per cent as much vitamin A as the sample cured in the dark.

Fresh third cutting alfalfa hay cocked, cured and not sweated in a stack contained 308 rat units of vitamin A activity per gram (554). The same hay cured for three days in the swath and then cocked and sweated in the stack contained 116 rat units. The same hay cured one day in the swath and sweated in the stack contained 144 rat units. Sweating did not cause a very significant loss of vitamin A activity.

There was no loss of carotene when alfalfa was artificially dried but when air dried the loss was 67 to 88 per cent (425).

Russell and co-workers (418) showed that carotene disappears rapidly from freshly cut plant material. They observed a loss of 80 per cent of the carotene in alfalfa during the first 24 hours of drying in the field. Similar results were reported by Guilbert (182) and Vail and associate (480). Loss of the vitamin A occurred soon after cutting (107). Also, serious deterioration occurred when the hay was exposed to heavy rains, or to intermittent showers, followed by sunny weather. The damage was less when the hay was cocked than when it was cured in the swath. Hauge and Aitkenhead (207), Hauge (206), and Guilbert (182) presented evidence which indicated that the large loss of vitamin A potency during field curing of hay was due to enzyme action.

Dexter and Moore (100) determined the carotene content of alfalfa cured in the swath, windrow, and cock and found that there was no appreciable difference between the hay cured in the swath and windrow 10 hours after cutting but that the alfalfa cured in the windrow was higher in carotene from 19 to 120 hours after cutting. There was no advantage of cocked hay over that cured in the windrow in carotene content at the end of 72 hours. Similar results were reported by Bartlett and associates (27).

Fagan and Ashton (132) did not observe a decline in carotene in grass after wilting for 24 hours in the field and only a small decrease after 96 hours. The weather during this time was ideal.

It was found that partial field drying or wilting for prolonged periods under unfavorable climatic conditions may destroy as much as 66 per cent of the original carotene. Much larger amounts of carotene are lost when herbage is fully exposed to sunlight than when wilting takes place in the shade. Rain accentuates the loss of carotene after 24 hours.

Fraps and Kemmerer (149) reported that carotene in alfalfa meal was more stable when stored at 6° C. than it was when stored at room temperature. Carotene losses from alfalfa meal stored in pint or quart jars at room temperature varied from 8 to 70 per cent in eight weeks, while at refrigerator temperature the loss varied from 0 to 26 per cent. When alfalfa meal was packed in homeopathic vials at a temperature of 35° C., it lost carotene rapidly at first, then quite slowly, indicating that some of the carotene may be easily destroyed while a portion may be protected in such a way that it is much less easily destroyed.

Taylor and Russell (462) reported that chopped artificially dried alfalfa hay, stored in bags in a barn, lost 50 per cent of its carotene during the first three months of storage (late summer and early fall). There was no loss of carotene during the winter but a further loss of 25 per cent occurred during the succeeding summer season. Storage of ball-milled, artificially dried alfalfa hay in vacuo in the dark at 0°–5° C. preserved the carotene very efficiently for 20 months. Both the exclusion of air and a low temperature are important factors in carotene preservation. The degree of fineness was of little importance. Very little carotene is lost during dehydration providing the drying temperature is sufficiently high.

Alfalfa leaf meal lost 50 per cent of its vitamin A in 11 months (154). Miller and Bearse (337) observed some loss of vitamin A when dehydrated grass, dehydrated alfalfa and sun-cured alfalfa were stored for one year, the rate of loss being greater with the grass than with the alfalfa. Kane and associates (163) showed that the loss of carotene in hay varied with the storage temperature.

Although as a rule the estimation of the color of the hay is an evaluation in terms of carotene, however, hay may have the good color of U. S. grade No. 1 and yet may not be high in carotene because chlorophyll is more resistant to change than carotene. The hay grade, therefore, is only a rough index of its carotene content.

Silage.—The conservation of carotene in silage making has been discussed under the preservation of nutrients. In view of the great losses of carotene during the curing process (ordinary methods) and the loss in storage, the silo appears to be one possible method of solving this problem. There was no loss of carotene when alfalfa and soybean plants were ensiled by the A. I. V. method (381). Watson and Ferguson (509) gave the carotene content of molasses silage and A. I. V. silage, on the dry basis, as 49.8 and 57.1 milligrams per cent.

Timothy silage was found to contain more carotene on the dry basis than the best dehydrated alfalfa hay (42). The high carotene content is of importance in the production of winter milk with a high vitamin A and color content. Silage made from grass was just as high in carotene as the fresh grass (40).

According to Woodward and Shepherd (558) an outstanding characteristic of the silages as compared with the hays was their superior content of carotene. These investigators preserved the carotene in the fresh, green, untreated alfalfa and grasses which they ensiled. The outstanding result of this investigation was the high preservation of carotene in grass and legume silage made without any treatment. The addition of inorganic acids or molasses did not conserve the carotene any more effectively than no treatment. In the 1936 trial, first cutting alfalfa in the fresh state contained 164 micrograms of carotene per gram on the dry basis, while no treatment silage, acid silage, molasses silage and field cured hay contained 175, 168, 112, and 16 micrograms of carotene per gram of dry matter respectively. Other workers have also reported the carotene content of other grasses and hays (216, 173, 425).

TABLE 27

Carotene content of feeds (322, 529)

Feed	Water	Carotene per gram of dry matter		
		High	Low	Average
	per cent		*micrograms*	
Fresh green Kentucky bluegrass	68.4	620	424	522
Fresh green corn plant, cut for ensiling	78.1	115	70	92
Corn fodder, old and dry	9.1	6	2	4
Corn silage	73.7	60	13	39
Wheat straw	8.4	...	...	2
Clover hay, U. S. Grade No. 1	...	43	11	23
Corn stover (dry)	...	6	2	4
Dehydrated alfalfa leaf meal	...	...	...	33.6
Cocksfoot (132)	...	...	...	33.0

Carotene Requirement of Dairy Cattle.—Reproductive failures in cattle associated with the feeding of roughage of poor quality were formerly attributed either to a calcium deficiency or to the so-called injurious effects of cottonseed meal. In the early studies with low calcium rations, the roughages used were oat or wheat straw, cottonseed hulls or timothy hay of poor quality. The work of Reed and Huffman (402) showed that reproduction was normal when timothy of high quality, from the standpoint of greenness, was fed along with corn silage as a roughage. These results were substantiated by Hart and co-workers (195).

Cottonseed meal injury in cattle, which had been attributed to gossypol, was shown to be due to the lack of the factor present in good hay (403, 242). Blindness was observed among calves when wheat straw was fed as the only roughage. This type of blindness was associated with a constriction of the optic nerve where it passed through the optic foramen (403, 347). Recently this type of blindness in calves was shown to be due to a vitamin A deficiency (346).

The relation of the quality of roughage to cottonseed meal injury in cattle has been studied extensively by Curtiss and associates and more recently reviewed by Curtiss (88). The relationship of the quality of roughage to cottonseed meal injury was also studied by Kuhlman and co-workers (286) and Cunningham and Addington (86).

Reproductive failures under natural conditions due to a deficiency of vitamin A in the roughage have been reported by Hart and Guilbert (200). Converse and Meigs (74) and Meigs and Converse (325) also associated the birth of weak and blind calves with the feeding of hay low in carotene.

Converse and Meigs (75) reported that when the ration contained more than 86 micrograms per pound of live weight, normal reproduction occurred, but when less than this amount was present, reproduction was not normal. On this basis, a 1,000 pound cow would require 86,000 micrograms of carotene daily. If such a cow consumed 20 pounds of hay containing 10 micrograms per gram, she would receive sufficient carotene to meet her requirements for reproduction. Since the average hay usually carries 15 to 20 micrograms of carotene per gram, there appears to be meager likelihood of reproductive failure occurring under farm conditions except in extreme cases. Also, cows receiving fall pasture usually have a good storage of vitamin A at the beginning of the dry feeding period which tends to reduce the possibility of a vitamin A deficiency from the standpoint of reproduction (150, 183).

The carotene requirement for normal growth is about 14 micrograms per pound of live weight (184). The vitamin A storage in the new-born calf is low (183) but ordinarily the colostrum contains sufficient amount of this vitamin to give the calf a start. Stewart and McCallum (451) determined the vitamin A content of the colostrum from 100 cows and found that it varied from 3.5 to 118 international units per cubic centimeter. The vitamin A content was also found to depend upon the length of dry period. Kramer and associates (283) reported that the colostrum of the first and second days of lactation contained 25 to 28 international units of A per gram for cows receiving supplemented rye pasture and 16 and 20 units for two cows receiving the basal ration. Similar results were reported by Dann (92). A calf weighing 88 pounds would require 2,000 international units per day. It appears from these data that ordinary colostrum would supply sufficient vitamin A for the first few weeks of a calf's life. The requirement during this period has not been adequately investigated. Converse and Meigs (75) are studying this phase of the problem.

Dahlberg and Maynard (90) and Keyes and associates (270) found that calves fed a good practical ration did not benefit from the addition of a cod liver oil supplement. In their calf starter studies, Savage and Crawford (422) reported favorable results when a fortified cod liver oil was used.

When good hay containing 20 micrograms of carotene per gram is used for calf feeding, one-fourth pound of such hay would meet the requirements of a 100-pound calf. It is apparent that under conditions of good calf management a vitamin A supplement is not necessary, with the possible exception of the first few weeks of a calf's life when whole milk is not fed.

Roughage and the Vitamin A Content of Milk.—The relation of carotene in the roughage to the vitamin A content of milk is of interest from the standpoint of human welfare because milk is the principal source of this factor in the human dietary.

The early work on this subject was reviewed by Hilton and associates (225). These workers showed that butterfat produced by cows on pasture was high in vitamin A. Under winter conditions, timothy hay produced a butterfat of low vitamin A potency while good quality alfalfa hay and soybean hay were effective in maintaining a high vitamin A level equal to pasture feeding. Baumann and Steenbock (28) showed that the carotene and vitamin A potencies of milk were higher during the pasture season and then gradually decreased during the winter feeding period. Hilton and co-workers (226) observed that cows fed artificially cured alfalfa produced milk higher in vitamin A than when fed field cured alfalfa.

Soybean hay made from plants after the beans were well formed suppressed the formation of vitamin A in the butterfat sufficiently to produce a butterfat of only medium high vitamin A activity. Soybean hay made from young plants failed to show this effect when fed to milking cows (226).

The vitamin A content of butterfat increased markedly within two weeks after turning the cows out to pasture (150). The apparent vitamin A recovery in the milk was 47 per cent for a cow receiving 8,432 units of vitamin A per day, 21 per cent for a cow receiving 60,189 units and 10 per cent for a cow receiving 116,101 units daily. The relationship of carotene in the ration to the vitamin A potency of milk has been investigated further by various workers (470, 151, 234).

The effect of feeding various types of silages on the vitamin A content of milk was investigated by several workers (380, 284, 509, 379, 216, 203, 234). In most instances the results of these investigators indicate that the vitamin A potency of the milk was of the same order as the carotene content of the roughage.

ROUGHAGES AS A SOURCE OF VITAMIN D

Green forage (44, 246) or artificially dried hay (33) were practically devoid of vitamin D. According to Steenbock and co-workers (450) the antirachitic potency of hay can be increased by exposure to solar radiation or ultraviolet irradiation. Three pounds of sun-cured timothy hay protected calves from rickets to one year of age and gradually increasing the hay to six pounds daily prevented rickets to 2.5 years of age (246). Two pounds of sun-cured alfalfa hay were sufficient to protect calves from rickets up to 195 days of age.

Corn cut for silage at the usual stage possessed antirachitic qualities (36). The daily ingestion of 7.0 to 10.0 grams of dry corn silage per kilogram of body weight cured and prevented rickets in yearling calves. The green part of the corn plant was free from this factor while the silks, tassels, and dried leaves were good sources. The corn silage contained 122 to 165 international units of vitamin D per pound of dry matter. The tassels, silks, and dry leaves of corn at the proper stage for ensiling contained 27, 54, and 5.4 international units of vitamin D per gram of dry matter respectively (34). The green part of the plant was devoid of vitamin D.

Bechdel and associates (33) found that 2.5 pounds of sun-cured alfalfa protected calves from rickets and that one pound prevented them from showing the clinical symptoms but failed to maintain normal blood inorganic phosphorus values. On the other hand, 2.5 pounds of dehydrated alfalfa hay failed to prevent rickets. Four to six pounds of prairie hay daily prevented the development of rickets in calves (500). Oat straw apparently contains appreciable amounts of vitamin D (33).

The vitamin D requirement of growing calves was from 1.0 to 1.5 international units per pound of body weight when corn silage was the only source of vitamin D (36). Similar results were secured when viosterol was used as the only source of vitamin in the ration (244). The results of Bechdel and associates (32) indicate a higher vitamin D requirement, namely, three international units per pound of body weight. On the basis of the higher requirement, a 200-pound calf would require 600 international units of vitamin D daily. On the basis of six international units of vitamin D per gram of alfalfa hay (498), only 100 grams of such hay would be required to meet the vitamin D requirement. In other words, calves which have access to sun-cured hay do not need a vitamin D supplement.

Although the amount of vitamin D in the milk may be influenced by the amount in the ration, the liberal feeding of sun-cured hay did not affect the amount of vitamin D in the milk appreciably (35, 499).

Roughage as a Source of Vitamin E.—Evans and Burr (126) reported that vitamin E was present in lettuce and alfalfa. Hathaway and associates (204) tested artificially cured and field-cured alfalfa hay for vitamin E using rats. Fifteen per cent of the ration as artificially cured alfalfa prevented resorption of the young, but the feeding of 15 per cent of field-cured alfalfa was no more effective than 10 per cent of the artificially cured hay. They concluded that artificial drying of alfalfa tends to preserve the vitamin E content to a greater degree than did field curing. In more recent work, these investigators found that 20 per cent of the diet of rats as alfalfa was effective in preventing resorption of the young (202). Zagami (563) reported that legumes furnished enough vitamin E for good reproduction in rats for three generations. Evans and Hoagland (127) found that etiolated and green seedlings of the Canadian field pea grown in an inorganic culture solution and also green seedlings grown in a solution of high sodium chloride content contained vitamin E.

The feeding of sprouted oats which are probably fairly rich in vitamin E benefited 57 females while 31 were not benefited (335). Henke (220) reported that out of 38 cows with irregular breeding behavior, to which sprouted oats were fed, 82 per cent produced calves and that out of 37 cows with a like history, which were not fed sprouted oats, 76 per cent produced calves. The evidence does not indicate that sprouted oats are beneficial in correcting breeding troubles.

Wheat germ oil which is the richest known source of this vitamin has been administered to cattle by several investigators. Unfortunately in most of these studies a check group was not used. Vogt-Moller and Bay (490) and Bay and Vogt-Moller (29) reported the results from treating a total of 70 sterile cows with wheat germ oil, of which 49 became pregnant. Risse (410) found that 10 out of 60 cows conceived within four to six weeks following treatment with wheat germ oil. Jones and Ewalt (259) injected wheat germ oil (10–20 cc.) into sterile cows. They reported favorable results in the case of 15, indefinite results in the case of 33 and negative results in five cows. They stated that no conclusions were warranted as yet. Tutt (476) stated that 17 of the 25 cows treated with wheat germ oil became pregnant and eight remained sterile.

Thomas and associates (466) showed that the goat does not require vitamin E. Whether or not the cow requires this vitamin remains to be proven. The evidence indicates, however, that when ample roughage of a good quality is fed a supplement supplying vitamin E is not warranted.

Roughage as a Source of Other Vitamins.—Recently Wendt (513) reported that milk from pasture-fed cows or cow receiving A. I. V. silage had a nutritive factor which stimulated lactation and the development of the nursing young. Peterson and associates (380) and Kohler and co-workers (280) observed a growth stimulating factor which was different from the known vitamins. This factor was called the "grass juice" factor. Henry and associates (221) failed to demonstrate a special "grass juice" factor in summer milk when compared with winter milk.

Although roughage is a good source of vitamins C, the so-called "vitamin B complex" and vitamin K, these factors will not be discussed in this review since cows apparently do not require them in their ration. Also, the presence of these vitamins in milk is not affected by their level in the ration.

THE UTILIZABLE ENERGY VALUE OF ROUGHAGE

Several methods have been worked out for the evaluation of the energy value of feeds in order to assign to each feed a single energy value. Kellner used "starch values," Armsby "net energy values," and Fraps "productive energy values," while "total digestible nutrients" has been used almost exclusively in the United States to denote a utilizable energy value. According to the first three systems, the net energy values for roughages are much lower than their total digestible nutrients indicate. Early experiments by Kellner showed that the digestible nutrients in roughages did not produce as much fat in the body of steers as did an equal amount of total digestible nutrients from concentrates. The productive value of the total digestible nutrients in the roughages used showed an inverse relationship to the crude fiber content. Consequently, in evaluating roughages the digestible nutrients were discounted according to the amount of crude fiber present. Associative effects of feeds in digestion and supplementary effects in metabolism were not considered. Several workers (50, 138, 141, 262, 315, 461) contributed evidence in support of the hypothesis that the utilization of metabolizable energy of a ration is a function of its balance with reference to the purpose for which it is balanced.

In other words, the utilizable energy value of roughages varies with the balance of the ration as well as with several other factors. Hamilton (192) using rats found that the growth-promoting values of diets increased as the percentages of protein increased from 4 to 18 per cent, that they remained constant between 18 and 30 per cent protein, and then decreased rapidly when 42 per cent or more of protein was present. Fraps (148) found that corn stover when fed with corn had a productive energy value of 12 therms per 100 pounds and 18.5 therms when fed with corn and linseed meal. Møllgaard (342) showed that when the percentage of net energy consumed as protein ranged from 15 to 25 per cent, the most efficient conversion of metabolizable energy into milk energy occurred.

Kleiber and associates (276) showed that a phosphorus deficiency depressed the availability of metabolizable energy about 10 per cent. Any deficiency which affects growth or milk production probably affects the productive energy of the feed or ration.

Grasses and legumes grown on well balanced soil and used either as pasture or hay, when the protein and digestibility were high, appeared to be about equal to concentrates on a dry basis (62, 360, 116). Such feeds also appeared to exert an energy value for maintenance and milk production about equal to their total digestible nutrient content. The results with a ration of alfalfa alone at the four United States Department of Agriculture substations in long-time feeding experiments likewise suggest that good quality alfalfa hay grown and cured under certain conditions manifests an energy value for milk production about equal to its total digestible nutrient value (171). In this work, however, the total digestible nutrient values were calculated but carbon and nitrogen balances are also needed in conjunction with such long-time feeding experiments in order to determine the productive energy value of alfalfa hay for maintenance and milk production.

Recent data (63) indicated that high producing dairy cows fed corn silage and a full allowance of a variety of high quality artificially dried hays, but no grain, produced milk as efficiently as cows fed the same quantity of corn silage, a smaller quantity of hay and a half allowance of grain. There was no difference in the efficiency of the conversion of total digestible nutrients to milk energy between the two groups of cows. Only two cows were used in each group.

Ritzman and Benedict (411) reported that one cow on alfalfa alone and one on soybean hay alone produced 10 to 11 kilograms of milk daily. The utilization of the metabolizable energy at this level of lactation was about the same whether the ration was deficient in energy or whether it supplied a surplus of energy. It was also the same when the metabolizable energy was derived from hay alone or when supplied by a mixed ration containing a substantial amount of grain.

The use of either five per cent casein or sucrose as supplements of a mixed herbage ration for rabbits resulted in changing a marked loss in body weight to a substantial gain (81). The deficiency was apparently due to a lack of available energy.

The results with alfalfa alone at Kansas (401), Oregon (258), and Michigan (245) suggest that alfalfa hay does not always manifest a productive energy value comparable to its calculated total digestible nutrients content. Haag (189) postulated that the low milk production on alfalfa alone observed in Oregon might be due to a deficiency of cystine. Rose (414) showed, however, that methionine rather than cystine is the essential amino acid containing sulphur. Cereal grains or beet pulp appears to furnish the essentials which alfalfa hay lacks (245).

Most of the recent data have indicated that hay cured under favorable conditions exert a productive energy value comparable to its total digestible nutrient content. When hay is made under adverse weather conditions variable amounts of the highly digestible nutrients may be lost because of their water-soluble properties. A variation of 10 pounds of total digestible nutrients per 100 pounds of hay would result in about a 60 per cent variation in milk production according to Morrison's requirements. This calculation is based on the assumption that available energy is the limiting factor in the nutritive value of roughages.

The vast amount of data which indicate that the total digestible nutrients of roughage are inferior to the total digestible nutrients of concentrates may be due to, (a) the stage of maturity of the roughage, (b) the loss of nutrients during the curing process, or both. The possibility that grasses and legumes, under certain conditions, may be balanced rations within the limits of the dry matter consumption should stimulate interest in this important economic problem.

ROUGHAGE AS THE SOLE RATION AND WITH VARIOUS SUPPLEMENTS

Pasture.—Although many investigators have studied the chemical composition and digestibility of grasses and legumes cut at various stages of growth, there is yet a paucity of data regarding the feeding value of such roughages when fed as the sole ration. Graves and associates (172) stated that if grass hay is of good quality, and is made from grass at an immature stage of growth, the grass hay alone will supply nutrients for the maintenance and production of somewhat more than 40 pounds of four per cent milk. In their experiments the cows did not consume enough grass silage to provide the nutritive requirements for their maintenance and the production of 30 pounds of four per cent milk but the rate of decline was slower on the silage. The results with grass clippings and grazing indicated that cows will consume enough of these feeds for maintenance and production of a large flow of milk.

Immature grass clippings, artificially dried, replaced a 20 per cent protein grain mixture in a 20-week feeding trial (62). The milk fat and solids-not-fat yields were about equal in each case, and on the basis of total digestible nutrients consumption, the dried grass ration proved to be as good as that containing grain. The substitution of artificially dried young grass for hay in a ration on a basis of equal amounts of total digestible nutrients resulted in an increased milk yield (360).

Elting and La Master (122) reported that substantial barn feeding was necessary to maintain profitable milk production on a pasture consisting largely of Bermuda grass, some Dallis grass and lespedeza, with carpet grass on the lower ground, and carrying slightly more than one cow per acre.

Geith (162) as a result of a study of some 30 dairies, some of which fed concentrates to cows on pasture while others did not, found that the average daily production per cow with concentrates was 35 pounds of milk and without concentrates, 34 pounds. He concluded that high, if not the highest, production can be secured from pastures alone provided they are properly managed.

The effect of turning cows out to pasture on the milk and fat production was investigated by Friis (160) of Denmark in 1899. Records were kept of the milk and fat content for three 10-day periods, preceding and following turning to pasture. These records covered 10 years and represented the production from 1,961 cows. While production rose immediately after turning the cows out to pasture, there was little difference in the average production although the fat content rose slightly.

Graves and associates (171) recently published results of a long-time feeding experiment in which the comparative values for milk production from hay and from silage were presented, each of which was made from immature pasture grass and fed as the sole ration for entire lactation periods. The pasture grasses from which hay or silage was made consisted of brome grass, or orchard grass, meadow fescue, white clover, perennial rye grass, Kentucky blue grass, and alsike clover. The four cows on the grass and hay ration produced an average of 11,306 pounds of milk and 403 pounds of fat in six lactation periods. Under full feed conditions, they produced on the average 14,958 pounds of milk and 528 pounds of butterfat. The four cows on the grass silage ration produced an average of 9,796 pounds of milk and 358 pounds of fat as compared to an average production of 13,207 pounds of milk and 498 pounds of fat under full feed conditions. Alfalfa hay was substituted for grass hay or grass silage for short periods when these feeds were not available. The decline in milk production during these periods supported the conclusion that the dry matter in immature grass hay or silage was superior for milk production to the dry matter in alfalfa hay which was cut at a stage of maturity in which one-half or more of the plants were in bloom.

Sixteen Jersey cows produced 6,333 pounds of milk and 329 pounds of fat in a year when fed a ration consisting of dried grass and hay. The same cows produced 9,656 pounds of milk and 527 pounds of fat in a year when the roughage was supplemented liberally with grain (167).

One group of cows on an all-roughage ration produced 342 pounds of butterfat yearly while another group which received grain (one pound per three pounds of milk) produced only 373 pounds (208). The all-roughage cows received 74 per cent of their feed supply from pasture while the grain group only received 52 per cent of their nutrients from a pasture under the same pasture conditions. A 4-year summary of two groups of 14 cows each, one with full grain (one pound to three pounds) and the other on limited grain (one pound to six pounds) was recently made (562). The group fed one pound of grain for each six pounds of milk (Jerseys) produced 97 per cent as much milk and butterfat as the full grain group and showed 11 per cent higher income over feed costs.

Wing (528) reported an increase in milk yield of cows for the first two weeks on pasture and a slight decrease during the next three weeks below the daily average for the last three weeks in the barn. Call and Fitch (61) observed an average increase in milk yield of 3.2 pounds of milk daily each time cows were turned on Sudan grass pasture. In an experiment with Ladino clover, significant increases in milk yield occurred when cows were turned from dry feed to fresh pasture.

Willard (522) studied the effect of pasture on milk production. The results are shown in Table 28.

Conserved Roughage Alone.—Woll and Voorhies (534) fed cows on alfalfa hay and silage during periods I and III and on alfalfa hay alone during period II. On alfalfa and silage the cows averaged 17.7 pounds of milk and 0.80 pound of fat, while on alfalfa hay alone they produced 14.1 pounds of milk and 0.63 pound of fat per day. An increase of 26 to 27 per cent in favor of the alfalfa hay and silage was observed in each case.

TABLE 28

Effect of pasture on milk production

No. of cows	Average daily milk production			Increase due to pasture
	Week before pasture	At peak on pasture	Difference	
	lb.	*lb.*	*lb.*	*per cent*
5	23.1	35.6	12.5	54.1
7	28.7	35.0	6.3	21.9
10	40.9	44.0	3.1	7.6

Woll (532) fed these heifers through two lactations on alfalfa hay and green alfalfa. In a later report (60), the following milk and butterfat records were given for all the lactations of the cows used, for alfalfa rations 6,491 pounds of milk and 259 pounds butterfat, for mixed rations 7,337 pounds milk and 323 pounds butterfat.

In addition to the above experiment eight heifers were fed alfalfa hay alone and another group of six heifers was fed alfalfa hay and succulent feeds—green feeds, silage and roots. The group on alfalfa hay alone averaged 6,375 pounds of milk and 223 pounds of butterfat, while the other group produced 7,240 pounds of milk and 257 pounds butterfat.

A herd of 23 cows in a California Cow Testing Association averaged 410 pounds butterfat in a year on alfalfa alone. There was no evidence that the exclusive feeding of alfalfa affected the breeding or fertility of the cows (532).

Reed and associates (401) conducted a long-time feeding experiment where one group of heifers was placed on alfalfa hay alone at six months of age and continued on alfalfa alone throughout the first and second lactations. Another group was placed on alfalfa hay and corn silage, while a third group was fed alfalfa hay, corn silage and grain. Although the appetite of the animals on alfalfa alone was good, their production was very low. The use of corn silage in place of part of the hay also resulted in low production. The production of the control group was considerably higher than the group on roughage alone. This group produced 70 per cent more fat during the first lactation and 101 per cent more fat during the second lactation than heifers fed alfalfa alone.

At the end of the second lactation two animals on alfalfa alone were switched to the regular herd ration. Cow No. 53 increased 119 pounds in butterfat production over the second lactation while No. 56 increased 155 pounds of butterfat. Live weight also increased about 200 pounds per cow during the third lactation.

Similar results were reported by Jones and associates (258) who fed six cows on alfalfa hay alone. Production was very low. The rapid decline in milk production on alfalfa alone suggested a lack of specific nutrients rather than of total digestible nutrients and these investigators questioned the biological value of the proteins of alfalfa hay. Wheat bran proved a good supplement to alfalfa. Huffman (245) placed cows on alfalfa alone at the time of calving. The cows made good use of the total digestible nutrients in alfalfa for about six weeks. At this point milk production showed a marked drop. A deficiency in the alfalfa was suggested inasmuch as a marked increase in milk production resulted when corn meal replaced an isodynamic quantity of alfalfa.

Jones (257) also reported that eight cows in Oregon fed alfalfa hay alone from one to three years produced 200 pounds of fat annually. No individual cow produced more than 278 pounds of butterfat in one lactation period although there were cows in the group that had had official test records up to 621 pounds of fat in a year. In a continuation of this work, Jones and coworkers (258) reported that average production on long hay was only 53 per cent of the average production on hay and grain. The low production on hay alone was attributed to the lack of a sufficient consumption of total digestible nutrients, and probably of phosphorus.

Alfalfa hay produced in central Oregon under irrigation was compared with that grown in the Willamette Valley without irrigation. One group received the central Oregon hay for 40 days and then after a transition period of 10 days was changed to Willamette Valley hay for 33 days. The second group was started on Willamette Valley hay and changed to the eastern Oregon hay. The results indicated the alfalfa from central Oregon (irrigated) was slightly superior for maintaining milk production. This was probably due to the greater consumption of this hay, 30 pounds per day, compared with 24 pounds daily for the Willamette Valley hay. Second cutting alfalfa of U. S. grade No. 2 was used in the Oregon investigation. Good milk production was usually maintained from four to six weeks after which a sharp decline was observed.

On the other hand, Headley (212) fed four cows for four years on alfalfa hay alone. This group averaged 304 pounds of butterfat annually. Their body weights remained fairly constant from year to year. This work was continued in which five groups were used (213): (a) on alfalfa hay alone, (b) alfalfa hay and grain, (c) alfalfa hay continuously and grain every other lactation period, (d) alfalfa at all times with silage in winter and pasture in summer, but no grain, and (e) same as (d) but with some grain added. The average annual production of cows on the all-roughage rations was 8,090 pounds of milk and 283 pounds of fat. The average annual production of cows receiving grain was 9,498 pounds milk and 331 pounds fat. Grain feeding resulted in 16.9 per cent increase in butterfat production and 17.4 per cent increase in milk production. He observed that butterfat production increased faster than the consumption of total digestible nutrients, thereby making the high-producing cows more economical producers than the low-producing cows. Headley listed the five factors which affect the profitableness of feeding grain as follows: (a) price of hay, (b) price of grain, (c) price of dairy products, (d) production of cow, and (e) quality of hay.

The feeding of grain to low-producing cows frequently did not increase the income above the feed costs when compared with cows on an all-hay ration. On the other hand, the feeding of grain to high-producing cows resulted in greatly increased production and, under favorable price conditions, a greater income over feed cost.

Seven hundred and two cows in 53 herds in a cow testing association in Nevada for the year 1922–23, fed alfalfa as the sole ration, averaged 7,060 pounds of milk and 263 pounds of fat (214). Cows may be expected to produce 70 per cent as much milk and fat on an all roughage ration as compared with a full grain ration (54). Willard (522) studied the effect of supplementing a roughage ration with grain. Two groups of cows were used. One group was given alfalfa hay and pasture in season and the other group was fed the same roughage but was fed ground barley at the rate of one pound to each five pounds of milk produced. Alfalfa hay was fed in a hay bunk. The alfalfa group produced 9,386 pounds of milk and 310 pounds of fat while the other group produced 10,180 pounds of milk and 323 pounds of fat per lactation period. The high production on roughage alone was probably due to high quality of alfalfa hay and the high character of the pasture used.

A study of a cow testing association's records of 18 herds, involving 214 cows on the Flathead irrigation project in Western Montana, revealed that 8 of the 18 herds, representing 57 per cent of all the cows, received no grain (256). The average production of these herds, fed roughage alone ranged from 200 to 300 pounds of butterfat annually and averaged 268 pounds. The 10 remaining herds which included 92 cows were fed an average of 3.2 pounds of grain per day. Their butterfat production averaged 305 pounds per cow. The difference cannot be attributed entirely to grain feeding.

Dickson and Kopland (103) reported the results of feeding 10 Holstein cows by three different methods: (a) full grain ration, one pound of grain for each three pounds of milk; (b) limited grain, one pound for each six pounds of milk produced; and (c) roughage ration, no grain. The cows were fed all the roughages they would consume. These consisted of alfalfa hay, corn silage, sugar beets, beet pulp, and pasture in season. Ten Holstein cows capable of high production returned an average of 464 pounds of butterfat during 365 days when fed roughage alone. Grain fed at the rate of one pound of grain to each three pounds of milk produced proved wasteful and decidedly uneconomical. One pound of grain fed for each six pounds of milk produced, proved to be an adequate and economical grain ration when fed with all the roughage that the cows could consume. These cows produced 94 per cent as much milk as did cows fed twice as much grain. It would require about 1.64 acres of alfalfa and 3.15 acres of grain to produce the feed required for one cow when given a full-fed grain ration (1 to 3); 2.27 acres of alfalfa and 1.49 acres of grain for a cow on the limited grain ration (1 to 6); while only 2.57 acres of alfalfa would supply all the digestible nutrients required for body maintenance and milk production for a cow on the roughage ration for one year.

A summary of the work of the United States Department of Agriculture, Bureau of Dairy Industry, has been published recently (173). Fifteen cows fed for a total of 24 lactations on alfalfa hay alone averaged 10,702 pounds of milk and 376 pounds butterfat per year. (The highest yearly record on alfalfa hay alone was 15,109 pounds of milk. This cow consumed 17,092 pounds of hay or one pound of hay per pound of milk). The same cows on full feed produced 15,342 pounds of milk and 538 pounds of fat. Seven cows that were fed the alfalfa hay ration for two consecutive lactation periods averaged 10 per cent less butterfat in the second lactation than in the first. The cows consumed an average of 14,352 pounds of alfalfa. They reached their highest average daily intake of 44.2 pounds during the fifth month of lactation. The highest individual daily consumption of hay was 69 pounds. Fifteen per cent of the hay offered was refused.

The results obtained by the Bureau of Dairy Industry with alfalfa alone are remarkable. The cows made efficient use of the nutrients in the roughage. The alfalfa used in the work was of good quality, most of it would have graded U. S. No. 1 alfalfa. Some was produced under irrigation and some was produced under dry-land conditions.

Alfalfa hay and corn silage as the sole ration was studied by Reed and associates (401) and has already been discussed. Frazer (155) conducted a 6-year home-grown ration demonstration with milking cows. Eleven cows on corn silage and alfalfa hay without grain produced on an average of 8,318 pounds of milk and 284 pounds butterfat in one year. They consumed 39 pounds corn silage and 15.3 pounds of alfalfa daily. The nine cows which were on the demonstration the longest time produced an average of 7,029 pounds of milk per year in 23 complete lactation periods without grain. The roughage was usually of exceptionally good quality and the cows consumed an average of 40 pounds silage and 16 pounds of alfalfa daily.

Dawson and associates (96) reported that, pound for pound, the dry matter in Sudan grass silage was more valuable in maintaining the milk yield than was the dry matter in field cured hay made from Sudan grass at the same stage of maturity.

Several investigators (156, 314, 236) showed that alfalfa hay was superior to timothy hay for milk production. On the other hand, other workers (402, 195) observed satisfactory milk production with timothy hay in comparison with alfalfa when the protein in the grain mixture was adjusted properly. The adverse effects of a timothy ration as reported by Meigs and Converse (325, 326) were due to inferior quality of the timothy fed. Maynard and co-workers (313) have shown that the nitrogen in timothy and clover hays is equally well utilized by milking cows. There was very little difference in the economy of conversion of feed energy of alfalfa and timothy rations into milk energy (140).

Recently Salisbury and Morrison (419) compared alfalfa and early-cut nitrogen fertilized timothy hay, using milking cows. There was no significant difference in milk or fat production, maintenance of body weight or in the percentage of hay refused.

In sections where alfalfa is adapted, it is the most economical roughage, especially in view of its greater yield of protein and digestible nutrients per acre. When plenty of alfalfa is consumed by dairy cows it is usually unnecessary to feed high protein concentrates. In sections where alfalfa can not be grown successfully, however, timothy and clovers are often used as roughages. The disadvantage of timothy hay in the past, other than its low yield per acre, has been the fact that it was cut at too late a stage of maturity. When dairy feeds are high in price the use of fertilizer may be indicated. Salisbury and Morrison (419) stated that 250 pounds of calcium cyanamid per acre of timothy cut in early bloom stage increased the yield 1,063 pounds of hay per acre in comparison with an unfertilized check. Protein was increased from 8.0 to 9.4 per cent by fertilization. Similar results were reported by other investigators (449, 396, 128).

Sprague and Hawkins (449) stated that high quality roughage from timothy hay and other grasses may be produced in any one of the following three ways: (a) growing mixtures of grasses and legumes which increase both the protein and mineral content; (b) harvesting grasses early when they are still high in protein, preferably grasses receiving nitrogenous fertilizer in the spring; (c) treating grasses at the early head stage with soluble nitrogenous fertilizers and harvesting 10 to 20 days later while the plants are still rich in protein.

Schultz (427) conducted experiments for several years which showed that it is possible to obtain satisfactory production without the use of oil cake and other concentrates. The basal ration consisted of meadow hay, marrow stem kale, silage of vetch mixtures, fodder, beet, and some lucerne hay.

Hodgson and co-workers (233, 232) divided 30 Holstein cows into three groups. One group was fed home-grown grass and clover hay, a second group received silage made from grass and clover and from oats and peas, and another group received a ration containing both hay and silage. The cows in all groups were pastured during the summer. No supplements other than salt and iodine were fed. Reproduction was normal in all three groups. The average butterfat production per year was 254, 251, and 258 pounds respectively for the three groups.

A ration consisting of 13 pounds of alfalfa and 30 pounds of corn silage was found by Lane (290) to be practical and economical in comparison to a ration in which two-thirds of the protein was derived from wheat bran and dried brewers grains.

Supplements to Roughages.—The extensive investigation of the Bureau of Dairy Industry on the feeding value of roughage alone indicate that profitable production may be secured without a concentrate supplement. In most regions in the United States, however, the pasture during the hot, dry months of July and August is of little value and should be properly supplemented. Likewise, the quality of hay available on the farms in many sections will probably not support profitable milk production without a concentrate supplement.

A home-grown ration of alfalfa hay, corn silage, and cereal grains will meet the protein and energy requirements of a Holstein cow producing 40 to 44 pounds of milk per day (196). It was suggested that 20 per cent of the grain mixture be either bran or linseed oil meal to insure against a possible phosphorus deficiency.

A ration of 16.4 pounds crimson clover and 30 pounds of corn silage proved practical when fed in comparison with a ration in which the protein was largely purchased. Also, 10 pounds of crimson clover hay, 36 pounds of cow pea silage and six pounds of corn and cob meal produced as much milk as a ration in which two-thirds of the protein was purchased in the form of dried brewers' grains and cottonseed meal. A ration consisting of 36 pounds of soybean silage, eight pounds of alfalfa hay and six pounds of corn meal produced more milk at less cost per hundredweight than a ration in which the protein was largely supplied by wheat bran, dried brewers' grains and cottonseed meal (291).

In a number of experiments, Billings (46) observed that crimson clover hay wholly replaced purchased proteins. Cow pea silage, crimson clover hay and corn and cob meal proved four per cent more efficient than mixed hay, corn silage and dried brewers' grains. Cow pea hay proved to be a good substitute for purchased proteins. Soybean silage, alfalfa and corn meal proved 11.7 per cent more efficient than a ration of corn silage, corn stalks, wheat bran, dried brewers' grains and cottonseed meal. When alfalfa replaced all of the purchased feed there was a drop of 4.1 per cent in milk yield which was probably not significant.

Caldwell (59) compared a ration of corn meal, corn silage, and alfalfa hay with corn meal, wheat bran, cottonseed meal, corn silage and corn stover, He concluded that alfalfa as well as soybean hay can replace much of the high-priced protein concentrates in the ration of dairy cows.

Horwood and Wells (241) compared a simple home-grown ration consisting of mixed alfalfa and timothy hay (equal parts) and corn with a complex ration consisting of mixed hay, sunflower silage, roots and a grain mixture consisting of five parts corn and one part cottonseed meal. There was no significant difference in milk production between these two rations. In another trial, a ration of alfalfa hay and barley was compared with a ration consisting of alfalfa hay, roots and a grain mixture consisting of 10 parts barley and one part cottonseed meal. The differences were not significant.

Hilton and associates (228) using alfalfa and soybean hays with home-grown grains failed to show a benefit from the addition of protein supplements.

According to True and associates (472), the addition of barley to an exclusive alfalfa hay ration gave only a slight increase in milk yield. When alfalfa hay and corn silage were fed liberally, the feeding of grain at a ratio of one pound of grain to five pounds of milk yielded within five per cent as good results as when the ratio was 1 to 3 (535). Experiments at the Massachusetts (297) and the Louisiana (302) Stations showed that full grain rations gave greater yields than rations in which part of the grain was replaced by roughage. The prices of milk and of the various feeds were considered important factors in deciding which system should be used for greater profit.

Monroe and Allen (343) compared a ration consisting of 30 pounds of alfalfa-timothy hay and 15 pounds of corn silage with one consisting of 15 pounds of hay and 40 to 45 pounds of corn silage. A small amount of grain was fed to the animals in each group. The cows on the heavy hay ration produced a little less milk but almost as much butterfat as did the cows on the light hay ration. Feed costs were lower and the returns over the feed costs were higher on the heavy hay ration. The light hay ration was compared with a moderate hay ration. Moderate hay feeding gave slightly greater production than did the heavy feeding but when the cost of purchased feed was considered, there was little difference between the two systems. These workers reported that the feeding of the heavy hay ration resulted in the retention on the farm of 95 per cent of the milk check, or seven per cent more than on the light hay ration.

The milk and fat production of cows fed roughage alone and with grain as reported in the literature is summarized in table 29.

The feeding of alfalfa alone gave variable results from the standpoint of milk production. The variation may have been due to (a) amount of hay offered, (b) climatic conditions during the growing season, (c) nutrients lost during conservation and storage, and (d) the inheritance for milk production. As has been pointed out the feeding of larger quantities of hay than cows will clean up, results in an increase in the quantity and quality of the dry matter consumed. The effect of climate and the losses during the curing process have been discussed.

Some of the high milk production records reported on rations of roughage alone may have been due in part to the liberal grain feeding during the previous lactation period. In a test of an all roughage system of feeding heifer calves should be placed on the roughage alone at 9 to 12 months of age.

The variable results with alfalfa hay alone indicate the advisability of supplementing the roughage with grain in order to secure economic milk production. The use of the old thumb rules for feeding grain, however, should be replaced by rules which permit the economical production of milk and fat.

TABLE 29

Feeding value of roughage alone and with various supplements for milk production

	No grain						Hay, grain with or without silage		
	Hay or silage			Hay plus corn sil.					
	Lact.	Milk	Fat	Lact.	Milk	Fat	Lact.	Milk	Fat
	days	*lb.*	*lb.*	*days*	*lb.*	*lb.*	*days*	*lb.*	*lb.*
Kansas alfalfa hay									
(401) 1st lact.	305	4,097	150	305	4,524	154	305	6,268	230
2nd lact.	305	4,151	150	305	4,351	161	305	6,044	222
Nevada alfalfa hay (212)	365	8,644	304	...	...	...	365	10,352	359
Montana alfalfa hay, (103) corn silage, pasture	365	13,295	464	...	...	...	365	17,851	544
Oregon whole alfalfa	305	4,421	166	...	...	...	305	7,208	278
(258) cut alfalfa	305	5,696	214	...	...	...	...	...	...
Calif.* 1st lact.	335	5,743	204	...	...	...	355	7,359	297
(532) 2nd lact.	354	6,372	286	...	...	...	302	7,420	351
U.S.D.A. alfalfa hay									
(173) 1st lact.	365	10,974	395	...	...	...	...		...
2nd lact.	365	10,239	357	...	...	...	...	...	...
Tenn. alfalfa, corn (208) silage, pasture	...	...	...	365	5,883	342	365	6,659	373
Ill. all roughage (155)	...	...	...	365	8,318	284	...	...	...
Wash. hay & pasture	365	7,953	254	...	...	...	...	...	...
(232) hay & silage	365	7,857	251	...	...	...	...	...	...
hay, silage & pasture	365	7,889	258	...	...	...	...	...	...
Wash. & alfalfa hay,									
U.S.D.A. silage, pasture	339	11,361	403	...	...	...	365	15,815	559
(232) alfalfa hay	359	10,702	376	...	...	...	365	16,255	521
(171) pasture grass hay	359	11,180	399	...	...	...	365	15,240	537
grass silage	353	9,796	368	...	...	...	365	13,207	498
artificially dried legume hay & pasture	337	6,333	329	...	...	...	361	9,656	527
Wyoming alfalfa hay, (522) pasture	386	9,386	310	...	...	...	404	10,180	323

* Alfalfa hay and fresh alfalfa.

RULES FOR FEEDING GRAIN

The trend toward more appetizing, nutritious roughages has necessitated a revision of the thumb rules for feeding roughage and grain. Frazer (155) formulated the following rules for economic feeding as a result of the more milk per acre demonstration:

(1) Full feed of corn silage and good alfalfa hay, or three and one-third pounds of silage and one and one-third pounds of hay per day per 100 pounds of live weight.

(2) For the larger breeds producing 3.5 to 4.0 per cent milk—one pound

of grain to each two and one-half pounds of milk produced above 20 pounds per day.

(3) For the smaller breeds producing 5.0 to 5.5 per cent milk—one pound of grain to each two pounds of milk produced above 12 pounds per day.

Headley (215) from the results on feeding alfalfa alone and alfalfa and grain suggested that Holstein cows giving less than 30 pounds of milk daily be fed good alfalfa alone but feed 0.4 pound of grain per pound of milk above 30 pounds per day. For Jerseys—no grain for cows producing less than 25 pounds milk daily and for those producing more than 25 pounds, feed one pound of grain for each 2.5 pounds of milk.

The grain feeding schedules to use with various qualities roughages are given by Morrison (350). These suggestions for grain feeding appear to be satisfactory.

SUMMARY

The nutritive value of roughages for dairy cattle depends upon the amount of dry matter consumed, the chemical composition and the coefficient of digestibility of the dry matter.

Dry matter consumption is affected by factors inherent in the animal and those inherent in the ration and environment. There is a wide variation in dry matter consumption of cows on the same roughage. The palatability of roughages is not a reliable criterion to use in estimating the amount which cows will consume. The trend toward the use of properly managed pasture, and hays and silage made from grasses and legumes cut at an earlier stage of maturity has resulted in an increase in roughage consumption per 100 pounds of body weight. Most cows will consume about three pounds of hay equivalent per 100 pounds of body weight which is a 50 per cent increase in roughage consumption over the old thumb rule for roughage feeding.

The chemical composition has been shown to vary with the stage of maturity, soil conditions and climate. Young plants are characterized by their high protein content, low cellulose and lignin content and a high digestibility of their dry matter. The decreased digestibility of the dry matter with increasing maturity is believed to be associated in some way with lignification although the total amount of lignin does not appear to be the determining factor. The greater use of properly managed pasture before the coefficient of digestibility drops makes the use of this class of roughage profitable. The data indicate that highly digestible roughage exerts a productive energy value about equal to that of concentrates on the dry basis.

The use of non-lignified grasses and legumes for winter feeding is very limited. This is due to the low yields and the difficulty encountered in the field curing of such roughages. Fertilization to increase the yield of roughage and its conservation in the silo after wilting make possible the use of highly nutritious roughage throughout the year.

The nutritive value of hays cut at the usual [illegible]urity depends upon the nutrients conserved during the cu[illegible]cess. As has been pointed out by several investigators, the loss [illegible]nts during the field curing of hay is rather high during good hay m[illegible]we[illegible]r but the loss of nutrients is very large during adverse curing [illegible]r. B[illegible]s of conserving roughages are needed because poo[illegible]king wea[illegible]s in many dairy sections. The cost of artificia[illegible]ration of hays appears somewhat high at the present time.

Recent experiments indicate that grasses and legumes may be conserved economically in the silo with a loss of only about 10 per cent of the dry matter. In several experiments, however, the loss of dry matter in roughage conserved as silage was rather high. Most investigations indicate, however, that the addition of from one to three per cent of molasses is advisable in the making of grass and legume silage. Inorganic acids may also be used to preserve these crops in the silo but molasses has the additional advantage of preserving the crop and contributing to the nutrition of the animal. Of the inorganic acids, phosphoric acid appears to have a slight advantage in silage making. It is easy to apply and it may contribute to the phosphorus requirement of the animal and to the fertility of the soil. Several investigators have reported that grasses and legumes make excellent silage without any treatment providing the ensiled material has a moisture content of about 70 per cent and the trapped air is expelled by fine cutting.

Many of the silage investigations, however, have been conducted either in small containers or in very large silos. In both cases the results may not represent the conditions in the average farm silo of about 50- to 100-ton capacity. In large silos the increased pressure may affect the moisture and carotene content of the silage as well as influence the capacity of the silo. There is very little difference in the dry matter capacity of the average silo for corn, grass or legume silage when the moisture and fineness of cut are about the same, but in large silos, more dry matter can be stored as grass or legume silage. Additional data is needed in regard to the conservation of grasses and legumes in the average farm silo.

Roughage protein appears to be well-balanced from the standpoint of the essential amino acids necessary for growth and milk production. The possibility of protein synthesis in the rumen by the rumen flora has been indicated.

Although grasses and legumes are probably very low in true fat, the results obtained with alfalfa alone suggest that when a liberal roughage program is followed there is little likelihood of encountering a fat deficiency. The possibility of a fat deficiency or of some factors closely associated with fat should, however, be considered.

The conservation of soluble carbohydrates in roughage is of practical importance because unfavorable weather conditions result in their loss in hay making. They may be of importance in maintaining the health of cattle duri[illegible] period. Ketosis has been shown to occur in cows fed ra[illegible]ble carbohydrates. This is explained on the basis of the [illegible] rumen digestion which postulates the conversion of foods w[illegible] sta[illegible] rumen to fatty acids by bacteria. The soluble carbohy-d[illegible] ha[illegible]ater chance of escaping bacterial action.

Grasses [illegible] legumes are excellent sources of carotene when used as [illegible]ture but whe[illegible]ed as hay, a large amount of the carotene is lost in field [illegible]ring and du[illegible] storage. The amount present in field cured hay is suffi-[illegible]t to mee[illegible] physiological needs of cattle for growth and reproduction [illegible] ro[illegible] is fed liberally. The conservation of carotene in roughages [illegible]ance in the production of milk high in vitamin A. Several investigators have reported the preservation of large amounts of carotene in grass and legume silage by the use of either molasses or inorganic acids. The results obtained by the United States Department of Agriculture, Bureau of Dairy Industry, indicate that a preservative is not necessarily needed for the preservation of carotene in grass and legume silages.

When green material is placed in the silo, the resultant silage is low in vitamin D inasmuch as this vitamin is formed in the cured plant material during solar radiation. The feeding of four or five pounds of sun-cured roughage per cow daily will meet the physiological needs of the animal for this vitamin during the winter months. Grasses appear to be good sources of vitamin E but whether or not cattle require this factor has not been ascertained. There is no evidence to indicate that the other vitamins in roughage are required in the ration of cattle.

Roughages are usually good sources of calcium and cattle have been shown to utilize about 50 per cent of the calcium in the ration when the supply of this element is rather low. There appears to be very little likelihood of a calcium deficiency occurring among cattle when they are allowed plenty of roughage.

Phosphorus is the mineral element which is most likely to be deficient in the ration of cattle when they are fed a heavy roughage ration because the phosphorus content of most roughage is low. The phosphorus content of roughage, under certain conditions, may be increased by phosphate fertilization but numerous instances have been recorded to show that the ordinary application of phosphate fertilizers to the soil did not influence the phosphorus content of the plant. The soil type and the moisture conditions are frequently the determining factors which influence the phosphorus content of the plant. In sections where the roughage is low in this element, the addition of a phosphorus supplement to the ration of milking cows is indicated.

Roughages grown on soils containing iodine are good sources of this element but on those farms where "big neck" or goiter occur among newborn calves, an iodine supplement should be supplied.

All of the other mineral elements required by cattle have been shown to be adequately supplied by roughage with the possible exception of a few localities where iron, copper or cobalt are needed as supplements.

The high production of dairy cows on roughage alone as reported by several investigators is of great practical importance and also of scientific interest. In these studies cows fed alfalfa alone appeared to make exceedingly good use of the total digestible nutrients for maintenance and milk production. Kellner, Armsby and Fraps have discounted the digestible nutrients in roughage according to its crude fiber content in arriving at "starch values," "net energy values," and "productive energy values." Apparently the low productive energy value of the digestible nutrients in the roughage studied by the many workers was due to some factor other than the crude fiber content. Alfalfa hay as the sole ration in several experiments, however, did not give results comparable to its total digestible nutrient content. In view of the variations in the productive energy values of alfalfa hay which has been reported in the literature, a grain supplement should probably be fed to cows in medium and high milk production. Rules have been suggested for feeding grain with roughage of good quality.

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American Dairy Science Association Announcements

RESULTS OF ELECTION

The results of the election of officers on October 1, were as follows:

Vice-President: **HARRY CAVE**, Oklahoma A & M College, Stillwater, Okla.

Directors to serve for three years each:

C. N. SHEPARDSON, Texas A & M College, College Station, Tex.

FORDYCE ELY, University of Kentucky, Lexington, Ky.

ANNUAL MEETING—1940—AT PURDUE UNIVERSITY, LAFAYETTE, INDIANA, JUNE 24–27TH

We trust that you are now making plans to attend the annual meeting next June. Those members who will have papers to read should write to the Chairman of the Program Committee whose name will be announced at an early date and inform him that they are willing to present a paper.

At the 1939 meeting in Pullman, Wash., and Moscow, Idaho, the Secretary was authorized by the Board of Directors to canvas the heads of dairy departments throughout the country to ascertain whether or not they favored a change in time of holding the annual meeting. These returns are at present being received. The Secretary's office would also like to receive opinions from the membership at large. If you have any criticisms of the present time of holding the annual meeting or any ideas opposing alteration of the date, please address a letter containing your opinions to the Secretary, so that the Board may have a representative group of expressions of opinion to consider.

TWENTY-YEAR INDEX

At our last annual meeting the Board of Directors authorized the indexing of our first twenty volumes of the Journal. This should be published within the next twelve months.

In order to make your Journals of greatest value we recommend that you have them bound by volume. The first twenty-two volumes contain practically all of the research work done in the field of dairying.

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A STUDY OF THE pH VALUES OF THE INGESTA OF THE BOVINE RUMEN

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The rumen, or first compartment of the ruminant stomach, plays a very important role in the digestive processes of the bovine species. It is here that the feed after being swallowed is stored, mixed, and softened with water and saliva, as well as subjected to a certain amount of digestive activity by bacteria. To maintain the optimum conditions for the bacterial activity, it is logical to assume that one of the requirements would be the maintenance of a proper hydrogen ion concentration. Although nature probably has a means of regulating the reaction in the rumen, it is probable that extremes in feeding have some influence. For instance, such extremes as heavy grain feeding or the excessive use of corn or A.I.V. silages may affect the balance.

LITERATURE

Dukes (1) states that "the reaction of the rumen content of the ox is always alkaline; the average is pH 8.89 (Gabriel)." He explains this constantly alkaline reaction on the basis of the addition of the highly alkaline saliva secreted by ruminants. An alkaline rumen reaction is noted by Stalfors (3) when the roughage fed was hay, straw, and oats bran or hay alone. Schwarz and Stremnitzer (4) found an average pH value of 8.28 in eleven animals that had been slaughtered. The day previous to slaughter these animals were fed exclusively on hay and straw. Kick and associates (2) working with steers having rumen fistulas report that "the pH of the ingesta varied from 5.5 to 7.7 depending on the ration." The most alkaline conditions were encountered when alfalfa hay alone was fed. They also report that there were variations in reactions during the day.

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[1] The authors wish to express appreciation for the advice and assistance given by Dr. A. F. Schalk of the Veterinary College of the Ohio State University, who is also affiliated with the Animal Industry Department of this Station. Likewise, credit is due to Dr. C. H. Kick for timely suggestions at the beginning of this work. Dr. Kick now deceased, was formerly, associated with the Animal Industry Department of this Station, and later was head of the Department of Animal Husbandry of Colorado State College. We are indebted to Mr. Stanley Powers of our Station for drawing the graphs.

METHOD

The samples of ingesta used in this study were obtained from animals in which a permanent rumen fistula had been established by surgical operation. Two such animals were used, one a Holstein steer approximately 18 months of age and the other a Jersey cow in her first lactation period. With the exception of having the fistula these animals were apparently normal. No special difficulty was encountered in maintaining the animals in a fairly thrifty condition. The steer gained in live weight as well as his feeding would permit, and the cow, although never a heavy milker, produced fairly well. She calved and conceived again during the course of the work. Both animals were kept in box stalls and bedded with wood shavings. When the experimental procedure called for it, they were turned out to pasture as normal animals. Neither animal gave any signs of undue discomfort when the samples were taken. The fistulas were kept closed by means of rubber plugs at all times except for the brief periods when the samples were being taken.

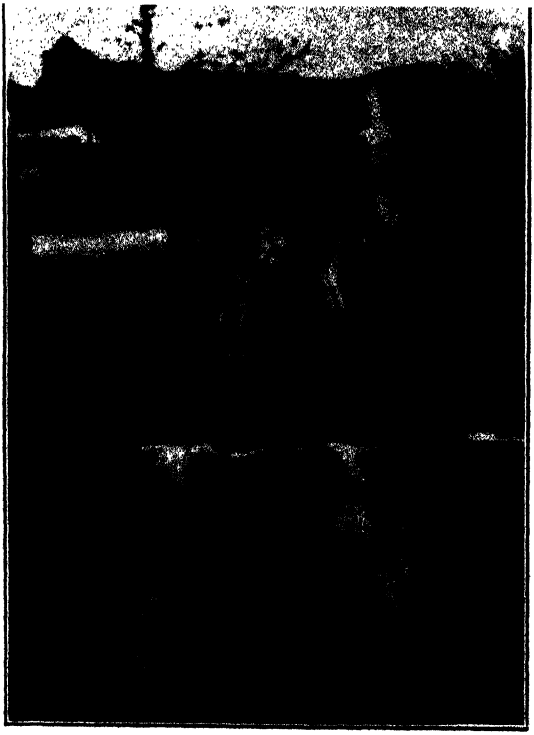

Fig. 1. Animals used in the experiments. The Jersey cow was bred and freshened during the investigation. The fistulas were kept closed by special rubber plugs which consisted of inner and outer flanges joined in the center by a core approximating the size of the opening.

In sampling the contents of the rumen six samples were taken each time. These were obtained from different locations as follows: three from the upper, or dorsal portion, distributed from front to rear, and three from the bottom, or ventral portion, from front to rear. Hereafter the top samples will be designated as front, middle, and rear, whereas the lower set will be designated as deep front, deep middle, and deep rear. The upper samples were taken approximately 1 or 2 inches below the surface of the rumen contents and the deep samples were taken at about the same distance from the floor of the rumen. In reporting the pH values, the average of the six readings is given unless otherwise specified.

When the samples were taken only once during the day, this was done at 1 P.M. Later, additional samplings were made at 2-hour intervals starting at 5 A.M. and finishing at 5 P.M. It was believed that this frequent sampling would tell a more complete story than the one-time-a-day sampling, although this latter method would have merit for comparative purposes.

Fortunately, as shown by the 2-hour procedure, the choice of 1 P.M. as a sampling hour was good, because at this time the pH reaction was close to the point of equilibrium for the day.

The pH values were determined on the uncentrifuged liquid expressed from the ingesta. All determinations were made on the fresh material within 1 hour or less after the samples were taken. The readings were made on a Leeds and Northrup portable potentiometer with a saturated calomel half cell and a quinhydrone electrode.

RESULTS

The pH values of the one-time-a-day samplings are shown in table 1. This table also shows the dates of sampling and the rations, together with the feeding periods. The average pH values of the urine are given in those cases where a sufficient number of urine samples was taken to justify an average. The data are classified according to the rations fed rather than in chronological order. It is believed that the animals were fed the different rations over periods sufficiently long for an equilibrium to be established.

When hay and grain were fed, the average pH value of the ingesta was 7.01. These results obtained in three different periods were fairly consistent. In the one period when both animals received this same ration the averages were very close together. The urine was consistently alkaline, averaging pH 8.29 for all the periods. In this feeding the amounts of hay given were either double or a little more than double the amounts of grain.

The addition of corn silage to the hay-grain ration and a slight reduction in hay did not result in a significant change in the pH values of the ingesta. The pH values on the hay-grain-corn silage ration averaged 6.95. Unfortunately, urine samples were not consistently taken on this ration, but those which were obtained at irregular intervals averaged a little over 8.00.

When A.I.V. silage having a pH value of 3.66 was substituted for the corn silage in the ration of the steer the pH value of the ingesta was little if any changed. The average was in this case 6.98. The urine pH value was lowered to around 6.35, however.

TABLE 1

The pH values of ingesta from various rations. Data from one-time-a-day samplings (Average pH values for urine shown in some cases)

Daily ration (pounds)				Experimental period	Experimental animal	Number of trials	pH values		
		Silage					Ingesta		Urine
Grain*	Hay†	Corn	A.I.V.‡				Range	Av.	Av.
				Days					
5	10	......	......	26	Steer	5	6.90–7.08	6.99	8.41
6	12	......	......	26	Cow	5	6.85–7.13	7.02	8.32
6	14	......	......	106	Cow	5	6.88–7.40	7.14	......
8	18	......	......	84	Cow	9	6.54–7.04	6.89	8.13
					Average for ration		......	7.01	8.29
4	4	12	......	39	Steer	10	6.74–7.16	6.94	......
4	4	12	......	29	Steer	5	6.81–7.03	6.91	......
6	6	12	......	29	Cow	5	6.75–7.05	6.93	......
10	14	20	......	32	Cow	4	6.91–7.20	7.03	......
					Average for ration		......	6.95	......
4	4	......	12	34	Steer	7	6.90–7.09	6.98	6.35
4	......	12	12	31	Steer	7	6.60–7.00	6.87	5.91
4	......	12	12	13	Steer	3	6.78–7.07	6.97	5.73
	(Plus 2 oz. limestone)								
10	......	68	......	47	Cow	10	6.58–7.10	6.83	6.59
10	Straw 2	56	......	14	Cow	5	6.76–7.04	6.90	6.36
5	Bluegrass pasture		......	28	Steer	8	5.91–6.86	6.44	......
6	" "		......	28	Cow	8	6.06–6.92	6.50	......
					Average for ration		......	6.47	......
6	Alfalfa pasture		......	48	Cow	5	6.55–6.85	6.66	......

* Grain mixture	Corn	500 lb.
	Oats	300 lb.
	Wheat bran	100 lb.
	Linseed Oilmeal	100 lb.
	Salt	9 lb.

† Hay, Second-cutting alfalfa.

‡ A.I.V. silage was made out of first-cutting alfalfa, containing some clover and timothy.

A still further change in the ration involving the substitution of corn silage for the hay made but little change in the reaction of the ingesta. On this ration, consisting of grain, corn silage, and A.I.V. silage, the reaction was 6.87. In the following period when 2 ounces of ground limestone were fed daily, the pH value was 6.97. The reaction of the urine on this ration with and without the supplemental limestone feeding was acid.

A ration of grain with corn silage as the only roughage gave a pH reaction of 6.83. The daily addition of 2 pounds of straw had but little effect. The pH in this case was 6.90. The urine was also acid on this ration.

On both bluegrass and alfalfa pasture the pH values of the ingesta were more acid than on any of the other rations.

An average pH value of 6.47 was obtained on bluegrass pasture and 6.66 on the alfalfa pasture. Although urine samples were not consistently taken, those that were taken when the animals were on the pastures show an average of around pH 8.00.

Two-hour sampling

All the results obtained in this phase of the work were obtained from the cow. The data are shown by means of graphs, in the accompanying chart. In addition, there is shown in table 2 a complete set of readings for one of the rations.

The table represents averages for three repetitions taken a week or more apart and after the animal had been on the ration a sufficient length of time to become adjusted to it. This is true for the other rations shown in the graphs with the following exceptions. The graph for the grain-corn silage-straw ration is for one day's collection and that for the grain and pasture is for two days' collection.

These curves, with the exception of the one on the pasture ration, show the same general trend. Before feeding in the morning, at 5 A.M., the reaction was at the most alkaline point of the day. After feeding there was a change to an acid condition which reached the low point at 9 A.M. There was then a gradual upward trend until 3 P.M., or just before the afternoon feeding. After this feeding there was another drop in the reaction.

The curve for the pasture-grain ration is much different from those for the other rations. The reactions show less variability during the day. From 5 A.M. to 11 A.M. there was little change. At 1 P.M. there was a slight drop in reaction, and then from 1 to 5 P.M. the readings were very close together.

The physical condition of the contents of the rumen was much softer and more liquid when the cow was on pasture than when she received hay and the silage combinations in the barn.

The values obtained by sampling every 2 hours during the day agree fairly well with those obtained by sampling once a day at 1 P.M. The agreement falls within 0.25 pH.

The trend of the reactions shown by the curve from an acid condition following eating toward an alkaline condition is probably the result of two factors. First, there is the copious secretion of alkaline saliva passing from the mouth into the rumen. Dukes (1) estimates that the ox secretes daily 56,000 grams of saliva. The same authority quotes figures of 8.1 and 8.23 as the pH value of this fluid. Samples of saliva taken from the experimental animals used in this work have shown a pH of 8.0 to 8.4. Second, there is the elimination of at least part of the acids, probably by their being dissolved out

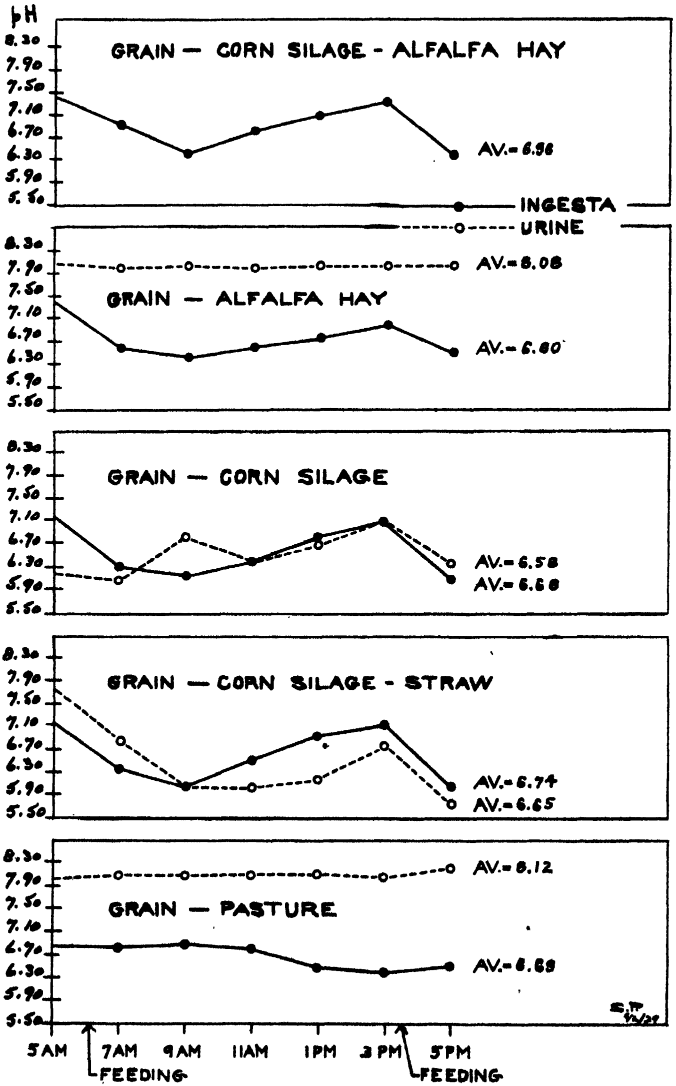

FIG. 2. The pH values of ingesta samples taken at two-hour intervals.

and passed along the alimentary canal with the more fluid portion of the ingesta. The change in urine reactions during the course of the day when acid rations were fed would indicate that this process goes on.

That the changes in reaction are the result of activity within the animal would be indicated by the following observations. Samples of ingesta taken at the 7 A.M. collection were kept in covered Mason jars in an incubator at

TABLE 2

pH values of ingesta, taken at 2-hour intervals. (Daily ration fed cow: 10 pounds of grain, 60 to 68 pounds of corn silage) (composite of three sampling dates, 2/23, 3/5 and 3/18)

Sample	Time							
	5 A.M.	7 A.M.	9 A.M.	11 A.M.	1 P.M.	3 P.M.	5 P.M.	Av.
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
Front	7.13	6.00	5.79	6.01	6.76	7.08	5.89	6.46
Middle	7.21	6.15	6.04	6.07	6.87	7.12	6.01	6.58
Rear	7.24	6.21	6.19	6.51	6.83	7.22	6.18	6.70
Deep front	7.17	6.51	6.37	6.80	6.90	7.06	6.23	6.80
Deep middle	7.21	6.48	6.40	6.60	6.91	7.09	6.37	6.78
Deep rear	7.19	6.42	6.39	6.42	6.86	7.03	6.33	6.72
Av.	7.19	6.30	6.20	6.40	6.86	7.10	6.17	......
Urine	6.27	6.18	6.82	6.38	6.70	7.10	6.32	....

Av. for Day—Ingesta, pH 6.68; Urine, pH 6.58.

100° F. for approximately 8 hours. A comparison of the pH values of this material after 8 hours with those of the original ingesta and of the material which had been allowed to remain in the rumen for this time showed that the acidity of the material in the jars increased rather than decreased. (See table 3.)

TABLE 3

Comparison of pH values taken at 7 A.M. and incubated for 8 hours with the same material allowed to remain in the cow

Sample*	pH values		
	At 7 A.M. (from cow)	At 3 P.M. (from cow)	Taken at 7 A.M. and incubated at 100° F. until 3 P.M.
1	6.92	7.24	5.17
2	6.83	7.29	5.36
3	6.98	7.13	5.47
4	6.30	7.31	5.71
5	6.39	7.02	5.51
6	6.09	6.39	5.54
7	6.37	7.12	6.40

* Samples taken on different days, various rations fed. Other samples taken at 9 A.M. and 11 A.M. showed the same trend.

pH reactions of different parts of the paunch

From the data presented in table 2, it will be noted that the reaction of the ingesta is not uniform throughout the rumen. This is probably a matter of mixing. The longer the material had been in the paunch the better it was mixed and the more nearly uniform were the reactions. For such a study the average reactions of the number of different readings have been compared.

The data in table 4 indicate that the reactions for the lower part of the paunch were fairly uniform and more alkaline than those of the upper por-

TABLE 4

Reactions of ingesta in different parts of the paunch at 5 A.M. *and 9* A.M. *(Data taken from 2-hour interval samples, pasture periods not included)*

Time	Front	Middle	Rear	Deep front	Deep middle	Deep rear	Av.
5 A.M.	7.23	7.36	7.32	7.28	7.32	7.31	7.31
9 A.M.	6.00	6.07	6.24	6.47	6.44	6.45	6.28
Av. for day	6.58	6.70	6.81	6.90	6.90	6.89	6.80

From one-time-a-day samples. Taken at 1 P.M.
(Same type of rations as above, but not same samples)

	6.85	6.94	7.02	6.98	6.99	6.99	6.96

tion. Of the six different locations, the front was the most acid. The variation in reactions ranged from 0.13 pH at 5 A.M. to 0.47 pH at 9 A.M. The range in the values for the average of the day was 0.32, and for the one-time-a-day samples taken at 1 P.M., 0.14 pH.

Slaughter data

An opportunity for obtaining the reactions on slaughtered animals occurred when it became necessary to kill a group of heifers and cows including cow 497—the Jersey cow used in this work. The animals were killed one at a time in a near-by slaughterhouse where it was possible by the aid of a portable potentiometer to make the pH determinations within 10 minutes following death. These animals had received no feed for a period of 12 to 18 hours previous to slaughter. The results of this study are shown in table 5.

TABLE 5

The pH values of the rumen contents of slaughtered animals

Animal no.	Age yrs.	Breed	Ration, pounds per day	pH values
346	11.0	Holstein	Grain 8; corn sil. 24; alf. h. 16	7.37
423	7.0	Jersey	" 8; " " 36; " " 8 and pasture.	7.30
497	4.0	"	" 4; corn sil. 20; alf. h. 10	7.42
509	3.5	Holstein	" 8; " " 20; " " 20	7.34
531	2.5	Jersey	...... pasture	7.34
534	2.5	"	Grain 6; corn sil. 14; alf. h. 10	7.25
537	2.5	"	...... " " 10 and pasture.	7.13
538	2.5	"	Grain 8; corn sil. 16; alf. h. 14	7.61
542	2.0	"	Alfalfa h. ad lib. pasture	7.30
544	2.0	"	" " " " "	7.50
545	2.0	Holstein	" " " " "	7.34
547	2.0	"	" " " " "	7.20
563	1.5	Jersey	" " " " "	7.42
564	1.5	Holstein	" " " " "	7.42
571	1.0	"	" " " " "	7.22
575	1.0	"	" " " " "	7.25

Average **Average of all, 7.34**
Older animals on grain, corn silage, and hay ration...... 7.35
Young animals alfalfa hay and late fall pasture.......... 7.33

The values obtained in the slaughter method are higher than the averages obtained by the fistula method, but they are only slightly higher than the values obtained at 5 A.M. in the 2-hour samplings. The slaughter data represent the same partially empty rumen condition as that found at 5 A.M. previous to the morning feeding. In fact an even longer interval had elapsed after feeding and before the cows were slaughtered than in the normal routine as represented in the 2-hour data. Just how much the excitement of moving and the strange quarters affected the reactions is impossible to state. Under these conditions animals are not as contented as normally and ordinarily ruminate but very little. Some appear to be more affected by the change than others.

SUMMARY

A study was made of the pH values of the contents of the bovine rumen. Samples of ingesta were obtained from animals having a rumen fistula.

The pH values of the ingesta averaged between 6.83 and 7.01 when such roughages as corn or A.I.V. silage and alfalfa hay in fair amounts were fed. On pasture, both bluegrass and alfalfa, the ingesta were a little more acid.

Samples taken at 2-hour intervals throughout the day showed the ingesta most alkaline just prior to feeding in the morning. After feeding there was a drop until about 9 A.M., and then a gradual trend toward alkalinity reaching a peak at 3 P.M. immediately preceding the evening feeding. After feeding there was again a drop in pH. This was for cows on winter rations. Those on pasture did not show these fluctuations.

The pH values were not the same in all parts of the rumen. The largest variations were found soon after feeding. At 1 P.M. these variations fell within 0.2 pH.

Samples of the rumen contents of animals slaughtered 12 to 18 hours after the last feeding had an average pH value of 7.34. These values were uniformly more alkaline than the averages noted above as taken from the living animals. They were, however, quite similar to those obtained at the 5 A.M. sampling in the 2-hour collection study.

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INJURY OR INFECTION IN UDDERS OF HEIFERS MAY TERMINATE IN BLIND QUARTERS

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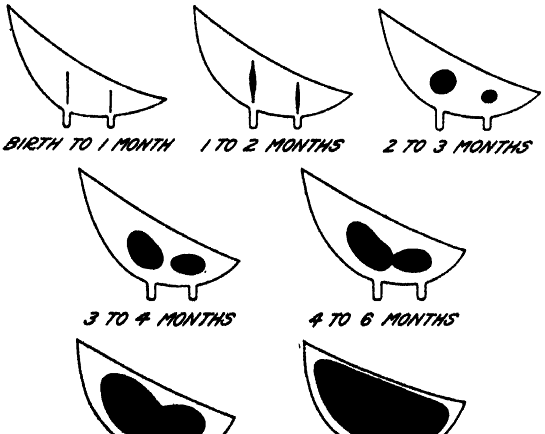
Marked reduction in, or complete cessation of milk secretion in one or more quarters of the udder, is not uncommon in cows. In some cases certain quarters are "blind" throughout the entire producing life of a cow. In some States and in some cities health authorities do not permit the sale of milk from cows having an inactive quarter regardless of the cause, and in some areas such cows are not permitted to remain in the herd for breeding purposes. A better understanding of the causes and the significance of such abnormalities is much to be desired.

It has been shown (1) that the four quarters of the udder develop from entirely independent tubular formations which are present and can be detected by palpating the udder of the calf at or soon after birth. These tubular formations which extend upward from the teats toward the abdominal wall first enlarge by bulging near the center. Later each of these elongated enlargements develops into a somewhat rounded formation which has no defi-



BIRTH TO 1 MONTH / 1 TO 2 MONTHS / 2 TO 3 MONTHS / 3 TO 4 MONTHS / 4 TO 6 MONTHS / 6 TO 9 MONTHS / 9 TO 18 MONTHS

FIG. 1. Diagrammatic illustration of the manner in which the mammary gland tissue develops in a calf.

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nite connection with the corresponding development in any other quarter until at usually about 4 or 5 months of age the front and rear quarters on the same side approach and come in contact with each other at the base and subsequently appear to be entirely fused together. The secretory and ductal systems, however, remain independent of each other through life. Figure 1 shows diagramatically what may be expected in the way of glandular development in the udder of a heifer of one of the dairy breeds at the ages given.

It has long been known, although perhaps not generally realized by laymen, that the four quarters of a cow's udder are entirely separate from each other and that normally the secretory system of one does not communicate with that of another—even the one on the same side. There is a distinct septum separating the right and left halves of the udder from each other. There is no visible septum separating the front quarter from the rear one although it has been demonstrated that fluid injected into the teat canal does not pass into any of the other quarters. Figure 2 shows a horizontal trans-

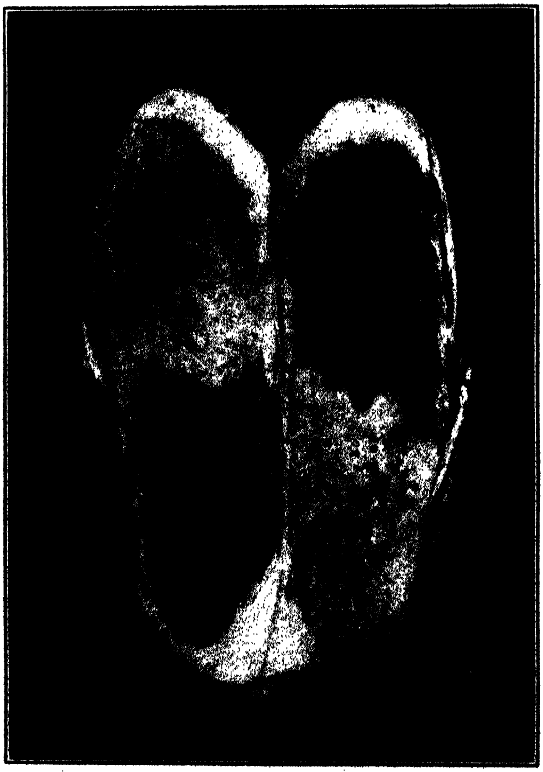

Fig. 2. Horizontal transverse section through an udder in which the right front and left rear quarters had been filled with a staining solution. Each of the quarters was separate from the others.

verse section through the udder of cow No. 50. After the udder was amputated, a staining solution was injected through the teats into the right front and left rear quarters. The other two quarters were filled with uncolored formalin. The section cut from the frozen udder shows clearly that the four quarters of the udder are entirely separate from each other.

Considering the independent development of the different quarters it is not difficult to visualize how one quarter can be injured or destroyed without the others becoming affected. Figure 3 shows a section through the two front quarters of an udder, the secreting activity of the left front quarter of which

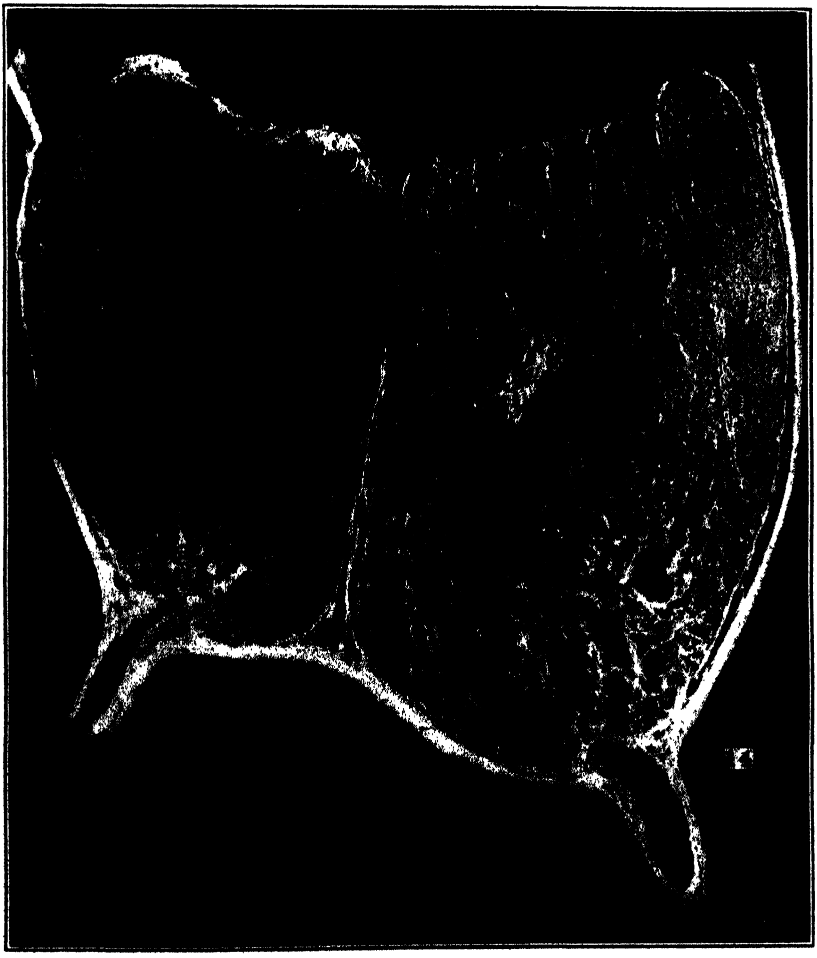

Fig. 3. Vertical transverse section through the front quarters of an udder showing that one quarter may become permanently "blind" without the other quarters being affected.

had been destroyed. The other three quarters apparently were functioning nomally.

The destruction of one quarter of an udder apparently does not necessarily impair the activity of the other quarters. In fact definite evidence has been presented elsewhere (2) to indicate that in certain cases where the loss of one or more quarters occurs early in life, there is a compensatory effect, which results in the remaining quarters becoming more than normally active in the secretion of milk.

Undoubtedly there are many different causes of "blind" or impaired quarters. Self-sucking or sucking by other calves, bruises and other injuries to the udder or teats, and mastitis, are perhaps among the most frequent causes. Many cases occur for which the cause cannot readily be determined. In the breeding herd of the Bureau of Dairy Industry at Beltsville, Md., it is the practice to make frequent examinations of the mammary gland development in calves, and of the udder structure through life in cows. Among the hundreds of udders studied over a period of years, there have been a number of cases of infection in one or more quarters that were contracted early in life. In nearly every such case the functioning of the affected quarters was permanently impaired or destroyed. Most of them failed entirely to secrete milk. Following are discussions of the history and performance of some of the observed cases, together with such photographic records as are available.

The youngest udder infection recorded is that of No. 1089. At the age of 18 days the left rear quarter was reported as being greatly enlarged and discharging pus from the teat. At 29 days the condition was essentially the same. At 1 month 28 days it was enlarged externally but the glandular development was somewhat retarded. The retarded glandular development in this quarter was marked until 4 months of age, after which the development became fairly uniform, and at 12 months was well above the breed average. When she calved no milk could be obtained from the left rear quarter. At an examination 3 days after calving the udder was swollen and no abnormality could be detected. Three weeks later, however, the left rear quarter was found to be light, stringy and apparently blind. As a 4-year-old she produced 8,808 pounds of milk and 537 pounds of butterfat from the three remaining quarters. This cow is still in the herd.

Another udder infection that occurred at a very early age is that of No. 1245. At 1 month of age the right front quarter was definitely enlarged and discharging pus. This calf was in an individual pen and could not have been sucked by other calves. Inquiry revealed the fact that the calf had jumped over the high concrete wall of her pen a few days before the condition noted above was seen. In doing so she may have bruised her udder. At 2, 3 and 4 months this quarter was definitely abnormal in development. At first it appeared enlarged and later was described as retarded. At 5 months and at subsequent examinations the udder development appeared to be fairly uniform in the different quarters and compared favorably with the breed average for development at most ages. After calving no milk could be obtained from either the right front or the left rear quarters. At the first observation after calving both of these quarters were found to be abnormal and it was soon apparent that they were "blind." There is nothing in the records to explain the reason for the failure of the left rear quarter to function. She was milked for about 3 months and then slaughtered. The best production for one month was 922 pounds of milk and 31.9 pounds of butterfat. Figure 4A shows the appearance of the gross anatomy of the two front quarters and 4B shows the structure of the two rear quarters at the time of slaughter. In the left rear quarter much of the tissue appears to be definitely fibrous and incapable of milk secretion. The small area of fairly normal appearance near the center of this quarter apparently was a part of the left front quarter

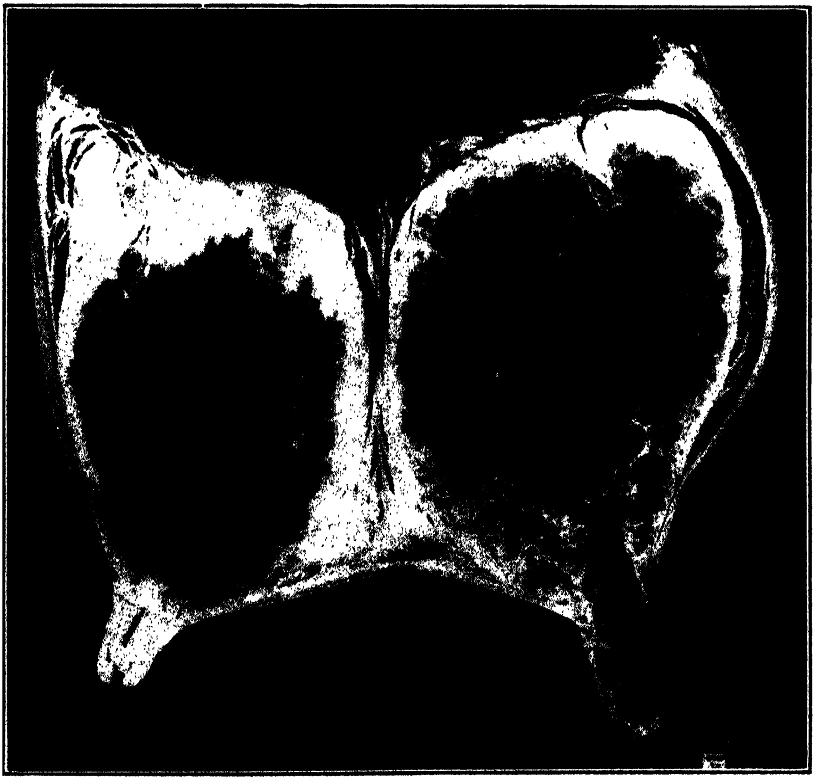

FIG. 4A. This photograph shows the destructive effects of active calfhood infection with pus discharge. The affected front quarter (at the left) resulted from an infection of the quarter at the age of one month. Note the fibrous infiltration in the teat and adjacent area. Rear quarters are shown in Fig. 4B.

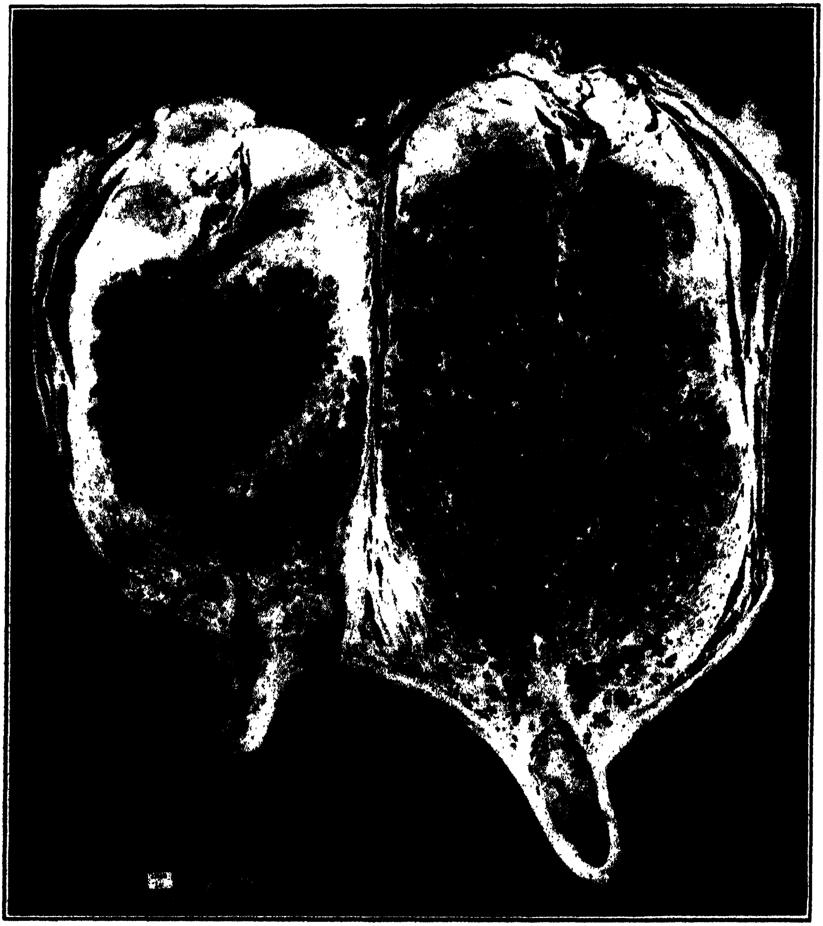

FIG. 4B. B, shows the structure of the rear quarters. The cause of the loss of the left rear quarter is not known.

that had invaded the left rear quarter territory. In the right front quarter, which appears at the left in figure 4A, the gland tissue appears to be more nearly normal, although actually it was woody. It is obvious that communication between the gland tissue and the teat canal in both the right front and left rear quarters had been cut off by the formation of fibrous or scar tissue. In all probability the gland tissue made a fairly complete development and secreted milk at calving time, but involution resulted soon thereafter because of inability to remove the milk.

The mammary gland development of No. 859 was not examined until she was 3 months of age. At that time the left rear quarter was definitely retarded and tubular in form. At 3 months 26 days the development was irregular. Subsequently up to 18 months there was nothing to suggest irregularity in that quarter. However, it differed somewhat from the others before calving. Milk was never obtained from the left rear quarter and soon after calving the quarter was obviously deficient. There is no record of infection or injury in this quarter although either could have occurred without detection prior to the first examination. The cow produced liberally despite the complete failure of the left rear quarter in milk secretion. The cow's record for 365 days at 6 years 2 months was 20,163 pounds of milk and 668 pounds of butterfat. Photographs of the gross anatomy of this udder are not yet available.

No. 882 was not examined for mammary gland development until the age of 4 months. At that time the right rear quarter hung lower than the others. At the age of 4 months 28 days the right rear quarter contained a long tubular formation "like a nail." A heavy fibrous growth running the entire length of the teat was noted at the 6-months examination. Nothing indicative of abnormality was recorded subsequently until shortly before calving when an unusually small opening from the teat to the cistern was noted. This quarter was non-functioning from the time of first calving. Soon the quarter became relatively light and stringy and was obviously "blind." Her production at 3 years 2 months was 11,919.9 pounds of milk and 439 pounds of butterfat. The udder was destroyed at the time of slaughter.

No. 881 was examined regularly beginning at the age of 13 days. At 4 months 2 days the left rear quarter was greatly enlarged, hard and discharging pus from the teat. The cause of the disturbance was not known. At the 5-months examination the same quarter contained a hard core that extended into the teat and at the 6-months observation the condition was essentially the same. At the 12-months examination the left rear quarter contained a distinct tubular shaped body of fiber that extended its entire depth. Before calving this quarter became enlarged, and intensely swollen. Following calving the quarter failed to yield milk. At the first examination after calving it was described as "blind but oversized." The cow's 365-day production commencing at 2 years 12 days was 9,391 pounds of milk and 341 pounds of butterfat. It is noteworthy that in this case, like that of 1245 the affected quarter became larger than the others before calving. Photographs of the gross structure of this quarter are not available but it appears likely that in this case also involution resulted from some obstruction that prevented removing the secreted milk.

The mammary gland development of No. 1001 was well above the average. No disturbance or abnormality was noted until the 5-months examination when the left front quarter was greatly enlarged, with the teat swollen and more than twice the length of the others. At six months it appeared that the disturbance had largely disappeared. Although there was no definite information to substantiate it the abnormality was attributed to sucking. No irregularity in this quarter was noted at 9, 12 or 18 months or at any of the four pre-calving examinations. The left front quarter was "blind" from the beginning of the first lactation. She produced 6,988 pounds of milk and 430 pounds of butterfat in 365 days, commencing at 2 years 1 month of age. When sections were removed for histological study after slaughter a hard lump was noted in the upper part of the left front quarter. Photographs of the gross structure of the quarter were not made.

Up to the age of four months the mammary gland development of No. 695 appeared to be uniform and above the average for the breed. At 5 months the right rear quarter was undersized, but no explanation was noted. At 6 months the left rear quarter was badly swollen, the teat was enlarged and was discharging pus. At 9, 12 and 18 months the development appeared to be uniform and above the average. Before calving there was no marked tendency for the left rear quarter to differ from the corresponding one on the right. However, it was "blind" from the beginning of the first lactation and soon became very small, fibrous and stringy. Production amounted to 9,756 pounds of milk and 581 pounds of butterfat at the age of 2 years 7 months. Photograph of the structure of this quarter is not available.

There was nothing notably unusual about the mammary gland development of No. 660 in its early stages. It appeared to be typical in form at 5 months and regular but slightly retarded at 9 months. At 12 months, however, the left front quarter consisted chiefly of a hard ball of fibrous tissue and the rear quarters were enlarged, ball-shaped and flabby. The udder showed indications of having been sucked. Subsequently abnormalities were not recorded until at an examination 2 days after first calving there was a "swelling in the teat" of the left front quarter. At a number of periodic examinations this same quarter was described in such a way as to indicate it was definitely abnormal and six months after calving it was described as "blind." The records do not show definitely whether the left front quarter failed entirely to function or whether it became blind soon after first calving. This quarter apparently did not function at any subsequent lactation. Apparently its failure resulted from the disturbance noted at the age of 12 months and there is reason to believe that sucking was the cause. Despite the possible handicap resulting from the disturbance noted she produced 12,885 pounds of milk and 678 pounds of butterfat in one year at the age of 7 years 1 month. The left quarters were used for histology studies and a photograph of their gross anatomy was not obtained.

The mammary gland development of No. 1428 was examined regularly beginning at 18 days of age. Grades for relative degree of development were high at each examination from 1 month to 12 months and no significant abnormality was noted. At the 18-months examination the left rear quarter was greatly enlarged and discharging an abundant quantity of thick, bright yellow pus. Although bred 17 times she failed to conceive and was slaughtered at the age of 2 years, 5 months, 22 days. Before slaughter the left rear quarter of the udder was greatly enlarged and, apparently full of fluid. This fluid amounted to 2.75 pounds and resembled milk. About 0.35 pound of colostrum-like material was removed from the right rear quarter. After the contents were removed the rear quarters were found to be fibrous and stringy in character. Despite her failure to conceive the gross appearance of the mammary gland tissue in the rear quarters, in which lactation apparently was abnormally stimulated in some unknown manner, closely resembled

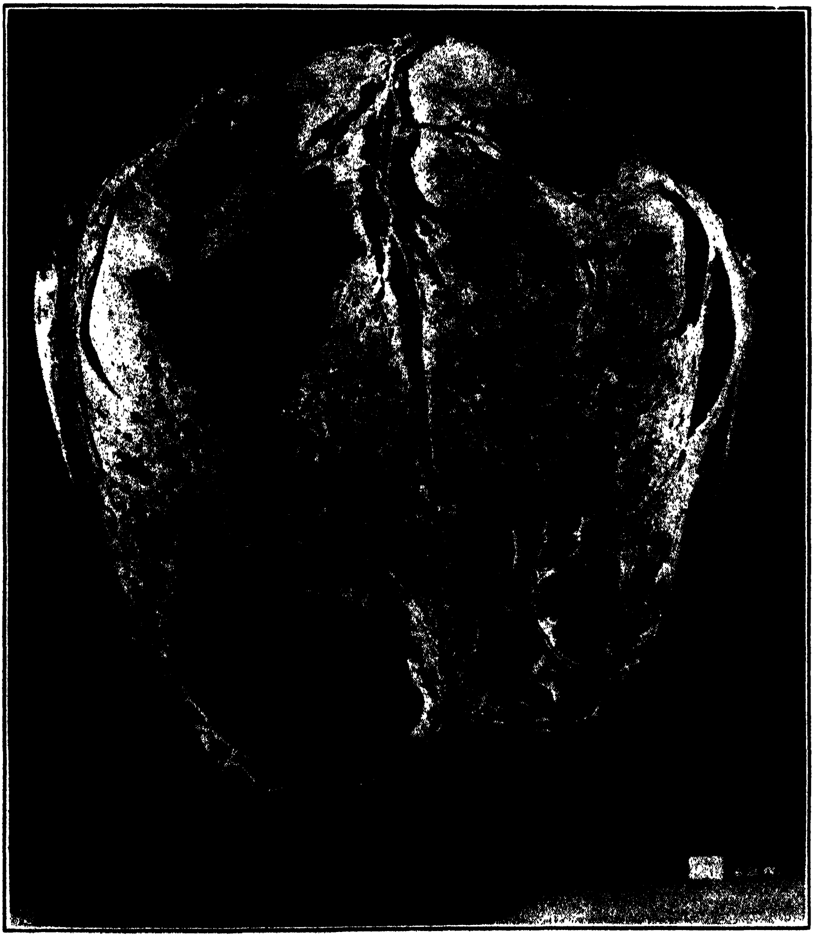

FIG. 5A. This photograph shows the structure of the two rear quarters of an udder in which the left rear quarter discharged pus copiously at 18 months of age. Fluid was found in both rear quarters when this heifer was slaughtered despite the fact she was a non-breeder.

that of a lactating cow. The two front quarters, however, consisted to a much greater extent of scattered areas of gland tissue imbedded in fat, a condition essentially typical of that generally found in heifers and cows that never conceived. Figure 5A shows the type of cisterns in the rear quarters and 5B shows the general structure of the front quarters.

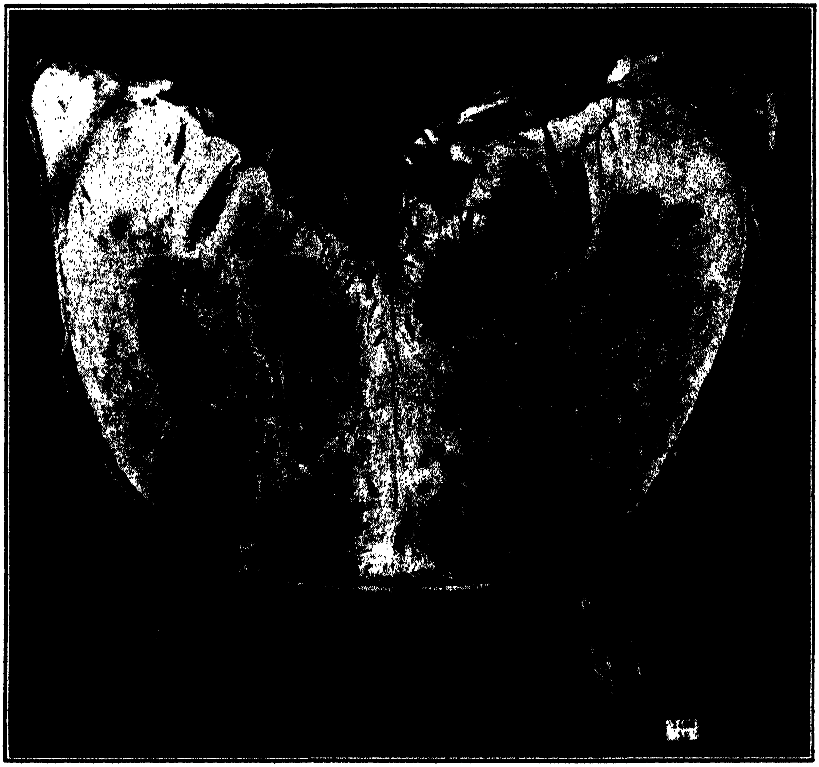

FIG. 5B. The mammary gland structure of the front quarters (rear quarters shown in Fig. 5A) which is fairly typical for a non-breeding animal that never conceived.

There was some indication of a retarded development in the right rear quarter of the udder of No. 1412 at the early age of 25 days, and at subsequent monthly examinations up to 5 months of age the right side seemed to be slightly smaller than the left. At 6 and 9 months no abnormality was noted and the development was above the breed average. At 12 months the right rear quarter seemed a trifle larger than the left rear but at 18 months no difference was recorded. The outstanding point about this case was a pus discharge from the right rear quarter approximately 3½ months before calving. The cause of this disturbance is not known. Two months after calving an indication of a deficiency in the size of the right rear quarter was noted.

At subsequent examinations the "lightness" of this quarter was consistently noted and the deficiency seemed to be progressive. Her record as a 2-year-old amounted to 10,071 pounds of milk and 625 pounds of butterfat. Approximately 4 months after second calving the milk yield from separate quarters was measured and it was found that only 10.3 per cent of her total milk yield was secreted by the right rear quarter when normally approximately 30 per cent would be expected. Here is a case where the infected quarter did not become blind but its functioning ability was greatly impaired.

No. 1438 showed no irregularity at any examination until at the examination made 20 days before calving, the right rear quarter was hard and lighter than any of the others and it was noted at the time that the quarter probably was "bad." On the day following calving it was found to be "blind," and 23 days later the same quarter was discharging pus. Figure 6 shows the appearance of the tissue in both rear quarters. Small areas of fairly normal appearing tissue may be noted in the nonfunctioning right rear quarter but connection between them and the cistern or the teat canal seems to have been destroyed so that milk, although present, could not be removed.

A more recent case is that of cow No. 1488. Her mammary gland development was better than average and fairly uniform until 18 months of age when the right front teat was described as higher than the others. At the examinations made 25 and 12 days before calving, the right front quarter was swollen, hard, and woody with a thickened condition in the teat. Milk has not been obtained from this quarter at any time.

A case of considerable interest is that of No. A 134. This heifer had a greatly enlarged udder at the age of 12 months when three of the quarters were filled out and discharging a milk-like fluid. Twelve days later the condition was even more extreme and the udder was very much distended. She was milked for about 2½ weeks after the appearance of fluid in the udder but the quantity of fluid obtained was not recorded. At the age of 21 months the three quarters were long and tubular, and apparently still contained milk. The udder was of extremely poor shape. At approximately 2 years of age the condition was more pronounced. The right front quarter was about 8½ inches deep and 3 inches in diameter and tapered into the teat. The right rear quarter was still longer and larger in diameter. The left front quarter was about 5½ inches long and 2¼ inches in diameter. The left rear quarter was almost entirely undeveloped. The enlarged quarters were cylindrical in shape except for tapering toward the teat. Each of the two right quarters appeared to contain an extremely large cistern but little glandular tissue. The left front quarter was more firm, harsh, and fibrous and its cistern appeared to be filled with a soft rubber-like substance. The cistern in the left rear quarter was very small and not readily detectable. She proved to be a non-breeder, and was slaughtered at about 24½ months of age. Her vaginal tract was found to be small and shortened, the uterus was in-

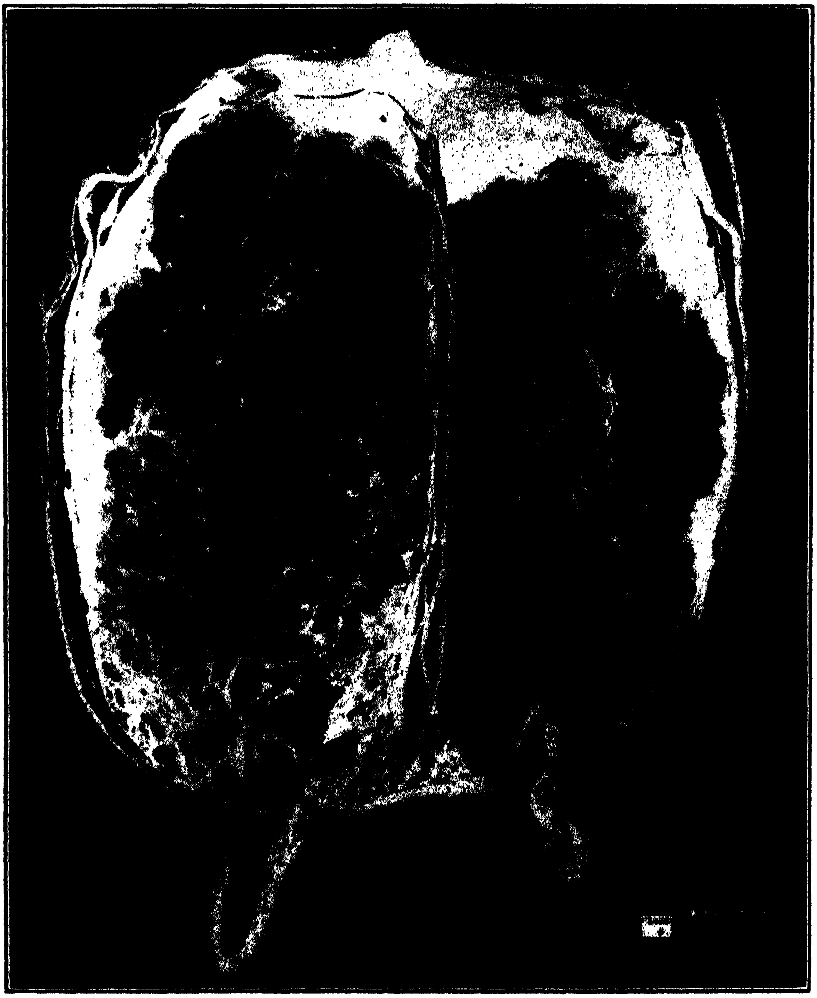

FIG. 6. Structure of the two rear quarters of an udder in which an abnormality was first noted 20 days before calving. The quarter on the right was "blind" from the time of first calving. Note the occlusion in the region of the cistern.

fantile, the ovaries were definitely abnormal, and one of the Fallopian tubes was occluded. The watery fluid milked from the two right quarters immediately before she was killed amounted to 3.47 pounds. Nothing could be obtained from either of the left quarters. The amounts of formalin injected into the four quarters were: right front, 1277 cc., right rear 2426 cc., left front, 59 cc., and left rear 59 cc. The formalin did not appear to penetrate far beyond the cistern in either of the two left quarters. The gross structure

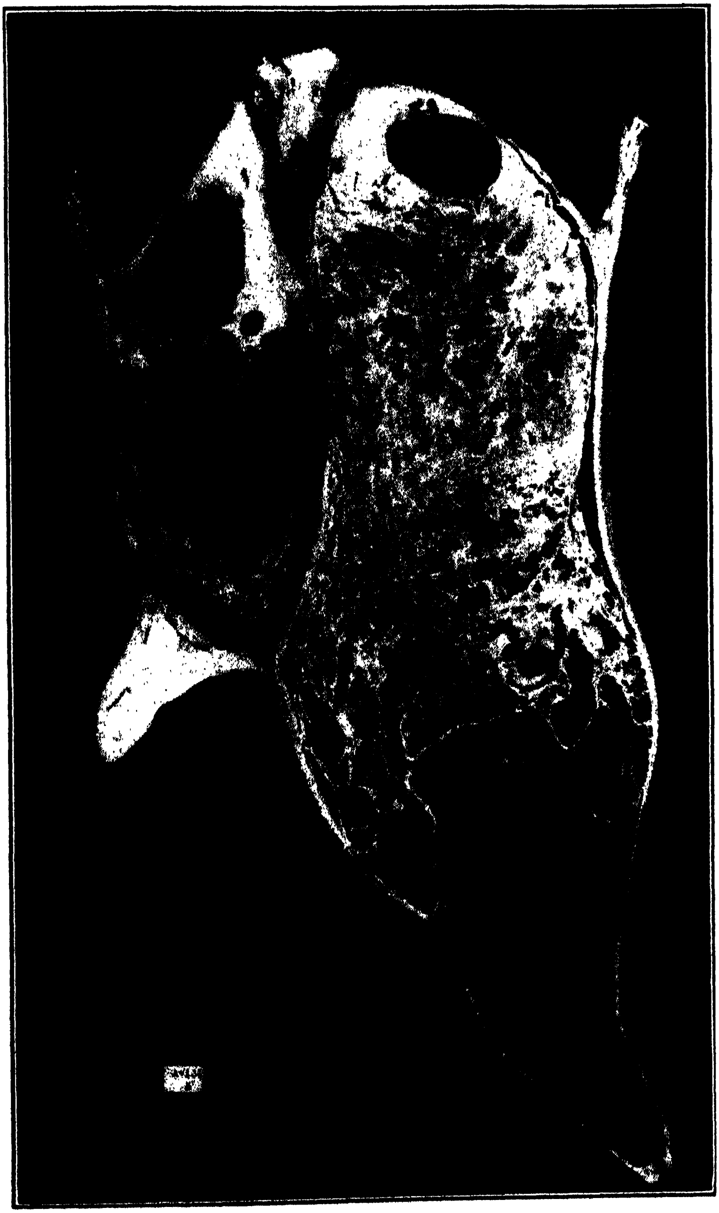

FIG. 7. Structure of tissue in the rear quarters of an udder in which the right rear quarter and both front quarters became distended at 12 months of age and remained so until time of slaughter at 24½ months. Note the enormous cistern in the affected quarter, a condition which is often found in cases of precocious activity of mammary glands.

of the rear quarters of this udder is shown in figure 7. It is suspected that this udder was sucked previous to its abnormal development at about 12 months of age. Nothing is known, of course, as to what the condition of the udder would have been after calving if this animal had been a normal breeder.

The cases described give some idea of the probable results to be expected from injury, infection or abnormal stimulation of the udder in heifers. Numerous cases of blind and deficient quarters of the udders have been observed following calving in cases for which there was no recorded history of disturbance in the calf. However, in every instance on record where pus discharge from the teat has occurred in the calf or young heifer, the affected quarter has failed to function from the beginning of lactation, except that of No. 1412, where the pus discharge occurred during late pregnancy and in that case the quarter became deficient after parturition. Pus discharge was not noted in the case of the non-breeding heifer No. A 134 but the appearance of the udder indicates that it was definitely abnormal and strongly suggests that its producing capacity would have been affected.

In nearly every case where udder infection has been found in the calf or young heifer, the affected quarters were abnormal in development for a time, but filled out approximately the same as the unaffected quarters before calving time. After calving, however, milk was not obtainable from these infected quarters and a marked decrease in size soon followed. In some of these cases, where post-mortem examinations were made, it appeared that there had been a development of secretory tissue and a development of fibrous or scar tissue in the region of the cistern. These findings make it appear probable that an early infection or injury does not necessarily cause a cessation in the development of the gland tissue in the affected quarter; that milk may be secreted in such quarters at calving time; and that occlusion of the outlet prevents removal of the milk thereby resulting in the involution of the tissue.

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COMPARATIVE VALUE OF NAPIER AND SUDAN GRASS WHEN USED AS SOILING CROPS FOR DAIRY COWS

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In areas adjacent to Honolulu and to other cities in the Territory, dairy cattle are commonly kept in small paddocks, although in regions remote from the cities, they are grazed the year round. In the paddocks the cattle receive their roughage from green soiling crops in racks. Concentrates are fed in the milking barn at the time of milking. The use of all available areas for growing soiling crops rather than for pasture is necessitated by the scarcity of the land.

Among various grasses grown in Hawaii as soiling crops, Napier grass (*Pennisetum purpureum*) and Sudan grass (*Sorghum vulgare sudanense*) occupy prominent places. The purpose of the investigation reported herein was to determine the relative value of these two grasses when grown as soiling crops for milking cows.

PLAN OF EXPERIMENTS

One general plan was followed in each of four experiments, with six cows selected for three experiments and eight cows for one experiment. The animals for each experiment were divided into two lots as comparable as possible with regard to milk production, body weight, age, days since calving, and date again due to freshen.

The reversal system of feeding was followed, and each trial was limited to 12 weeks to avoid the effects of advancing pregnancy. This reversal arrangement is clearly shown in table 1.

TABLE 1

Reversal system of feeding soiling crops

	Soiling crop fed	
	Lot I	Lot II
First 4 weeks	Sudan grass	Napier grass
Second 4 weeks	Napier grass	Sudan grass
Third 4 weeks	Sudan grass	Napier grass

The first week of each 4-week period was designed to accustom the cows to change of feed and results of only the last 3 weeks of each feeding period

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were used in computations. The averages of the first and third periods of each lot were compared with the second or middle period, thus compensating for decreasing production due to advancing lactation. The results reported for each experiment are again the averages of lots I and II, thus compensating for conditions which could not be controlled, such as temperature, rainfall, *et cetera.*

Ample protein and total nutrients based on the Morrison Standard were available for the cows in both lots in each of the four experiments regardless whether Sudan or Napier grass was fed. However, because of the higher consumption of Sudan grass and its higher protein content, protein intake was in greater excess of requirements when Sudan rather than Napier grass was fed.

Both lots of cows in each experiment were kept in small paddocks, except during milking hours. The roughages were fed in racks, and roughages unconsumed after 24 hours were weighed back. Concentrates were fed in the barn at milking time, twice daily. In each experiment all cows received the same concentrate mixture, but in quantities adjusted to the milk production of each cow during the first and second 4-week periods of each trial. During the last 4-week period, the quantity of concentrates fed each cow was adjusted so that the average consumption of the first and third 4-week period would equal that of the second 4-week period. Thus, a cow fed 15 and 14 pounds of concentrates daily during the first two periods, respectively, would be fed 13 pounds daily during the last period to avoid a variable other than roughage. The quantity of concentrates fed the third period

TABLE 2

Milk production, butter fat content, and weights of cows fed Sudan and Napier grasses

	Average daily milk production	Average butter fat content of milk	Average concentrates fed per day	Average green roughage consumed per day	Average live weight of cows
	Pounds	*Per cent*	*Pounds*	*Pounds*	*Pounds*
Sudan grass					
Experiment I	29.7	3.55	15.8	68.7*	996
Experiment II	31.0	3.38	16.6	58.5*	1,012
Experiment III	24.4	4.24	16.2	48.1†	997
Experiment IV	25.5	4.18	14.2	57.7†	1,018
Average	27.6	3.84	15.7	58.2	1,006
Napier grass					
Experiment I	27.8	3.68	15.8	63.5*	999
Experiment II	28.5	3.44	16.6	58.0*	1,008
Experiment III	22.3	4.38	16.2	45.0†	965
Experiment IV	22.9	4.31	14.1	50.9†	1,011
Average	25.4	3.95	15.7	54.3	996

* Roughages fed whole—only the finer parts of plant consumed.
† Roughages cut in ensilage cutter before feeding.

was generally in rather close accord with the needs of the cow based on her milk production.

Live weights of each cow were taken at the end of each week and averaged; butter-fat percentages were based on composite samples (four milkings), also taken at the end of each week for each cow. The summarized results are shown in table 2.

In each experiment milk production was higher when Sudan grass was fed, the difference averaging 8.66 per cent. While this is not a large difference, it was a consistent one, only 2 of the 26 cows in the 4 experiments producing more milk when Napier grass was fed.

Statistical analysis[3] consisted of calculating the differences between average daily milk productions of the individual cows when fed Sudan grass and when fed Napier grass, and determining the significance of the mean difference. The statistics are summarized below:

n = number of comparisons between Napier and Sudan grasses = 26
Sx^2 = sum of squares of deviations from the mean difference in production = 45.33
m = mean difference in production = 2.3 pounds
Sm = standard error of the mean difference $= \sqrt{\frac{Sx^2}{n(n-1)}} = \sqrt{\frac{45.33}{26(26-1)}}$
$= \pm 0.26$

$$t = \frac{m}{Sm} = \frac{2.3}{0.26} = 8.8$$

For a probability of 1 per cent with 25 degrees of freedom, a t value of 2.7 is necessary. The t value calculated above greatly exceeds the required amount, indicating that the difference in production due to feeding of Sudan grass was highly significant.

Percentages of butter fat in the milk averaged higher in each trial when Napier grass was fed, 19 of the 26 cows showing a gain in butter-fat content on this grass. The mean gain was 0.11 per cent, with a standard error of 0.0453. The calculated t value was 2.4 which indicated significance at the 5 per cent probability level.

The differences in live weight of the cows were not significant.

Consumption of green roughages as shown in table 2 was higher on Sudan grass, indicating that the greater value of Sudan grass for milk production was probably due to its greater palatability, resulting in larger amounts consumed.

In the first two experiments the green roughages were fed whole, immediately after cutting, and the portions weighed back after 24 hours consisted largely of the coarser parts of the stems. In the last two experiments the green roughages were put through an ensilage cutter prior to feeding. Samples were taken daily of the green feed as well as the portions unconsumed after 24 hours. Moisture determinations were made on all these daily samples, and composites of each 3-week period were analyzed for crude protein, ether extract, nitrogen-free extract, fiber, and ash, thus making it possible to determine actual nutrient intake in the roughages consumed. These data are shown in tables 3 and 4.

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[3] Snedecor, G. W. Statistical Methods, 341 pp., illus. Ames, Iowa, 1937. The help of Dr. J. H. Bartlett of the Hawaii Station in the statistical analysis of the data is gratefully acknowledged.

TABLE 3

Composition of Sudan and Napier grasses (calculated to a water-free basis)

	Crude protein	Ether extract	Nitrogen-free extract	Crude fiber	Ash	Total nutrients
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Sudan grass						
Experiment III	6.16	1.30	45.23	37.28	10.02	91.58
Experiment IV	8.37	1.13	42.32	36.35	11.83	89.59
Average	7.26	1.21	43.77	36.81	10.92	90.58
Napier grass						
Experiment III	4.76	1.12	40.08	38.86	15.19	86.22
Experiment IV	5.55	1.24	38.54	38.43	16.24	85.32
Average	5.15	1.18	39.31	38.64	15.71	85.77

TABLE 4

Nutrients contained in roughages consumed by cows in Napier-Sudan experiments III and IV (calculated to a per-cow-per-day basis)

	Weight of green forage consumed	Dry matter consumed	Total protein consumed	Total nutrients consumed
	Pounds	*Pounds*	*Pounds*	*Pounds*
Sudan grass	52.9	11.5	0.87	10.4
Napier grass	47.9	7.6	0.41	6.5

It appears from the above table that, since concentrates fed the cows were the same (see table 2), the lower milk production on Napier grass was due to the lower palatability and consequent lower protein and total nutrient intake.

SUMMARY

In four experiments comparing green Sudan with green Napier grass as a soiling crop fed to dairy cows, the former proved superior in each test on the basis of quantity of milk produced. Milk production averaged 8.7 per cent higher, and roughage consumption 7.2 per cent higher when Sudan grass was fed. The higher production on Sudan grass resulted from a greater nutrient intake because of greater palatability and higher nutritive value. Butter-fat tests were slightly higher when Napier grass was fed. Analyses of the grasses show that the Sudan grass was higher in protein, nitrogen-free extract, and total nutrients than Napier grass.

THE LIPIDS OF MILK. II. THE FAT ACIDS OF THE SPHINGOMYELIN-CEREBROSIDE FRACTION

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Phospholipids occur in all cells and to the extent of approximately 0.034 per cent in the milk of cows. Though their exact function in the animal body is not known, they have been thought to be intimately associated with fat synthesis. Although a minor constituent of food they may prove to be of especial significance.

Osborne and Wakeman (1) identified a monoamino- and, presumably, a diamino-phospholipid in milk, and their data indicated that oleic and stearic acids were constituents of the monoamino fraction.

Sasaki and Hiratsuka (2) concluded that the lecithin in milk contains myristic and lauric acids; and the cephalin of milk, palmitic and lauric acids.

Kurtz, Jamieson, and Holm (3) isolated the phospholipids from sweet cream buttermilk powder and separated them, on the basis of their solubilities in ether, into two distinct fractions: an ether-soluble lecithin-cephalin fraction, and an ether-insoluble sphingomyelin-cerebroside fraction. Their analysis of the lecithin-cephalin fraction showed that the fat acids consist of 5.2 per cent myristic, 16.1 per cent stearic, 1.8 per cent arachidic, 70.6 per cent oleic and 6.3 per cent of what was presumably a dicostetrenoic acid.

The analysis of the sphingomyelin-cerebroside fraction is presented in this paper.

EXPERIMENTAL

Isolation of material

The isolation of the phospholipids has been described fully in the first paper of this series (3). It consisted essentially in the extraction of three hundred pounds of sweet cream buttermilk powder with a mixture of 3 parts of ethanol and 1 part of gasoline. The extract was concentrated in a vacuum pan and then added to acetone. The precipitate which formed was freed from fat by a procedure involving repeated precipitations with acetone from ether solutions and from water emulsions and, finally, trituration of the precipitate with hot acetone.

During the purification of the phospholipids, as outlined above, it was observed that if an ether solution of the phospholipids was kept over night at 0° C., a light brown precipitate was formed. It was found that the solubility of this precipitate in the ether solution of lipids decreased as the lipids became more pure. Consequently, additional precipitates were obtained, at

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each stage in the purification, by holding ether solutions of the phospholipids at 0° C.

The ether-soluble lecithin-cephalin fraction weighed 580 grams. The ether-insoluble fractions were combined and together weighed 90 grams. This material was freed from contaminating lecithin and cephalin by crystallization from ethanol followed by washing with ether. This procedure was repeated until the originally discolored sticky mass became a white powder. The yield was 60 grams. The absence from its hydrolytic products of oleic acid, which forms 70 per cent of the acid constituents of the lecithin-cephalin fraction, indicates that it had been satisfactorily freed of these two phospholipids.

The material gave a positive orcinol test for carbohydrate. It contained 2.52 per cent nitrogen and 2.06 per cent phosphorus. These values are consistent with those for a mixture of sphingomyelin and cerebrosides in approximately equal amounts. When rubbed with concentrated sulphuric acid a deep purple color was produced. Thudichum (8) found that this reaction takes place when sphingosine and a sugar are treated with concentrated sulphuric acid. The material was practically insoluble in ether and in petroleum ether. Its solubility in 95 per cent ethanol at room temperature was 2 per cent; in hot ethanol, 13 per cent; and in chloroform at room temperature, 20 per cent.

Acids of the sphingomyelin-cerebroside fraction

Fifty-five grams of the material was hydrolyzed in methanol containing 10 per cent by weight of sulphuric acid, according to the method of Merz (4). Of the fat acid esters formed, 15.2 grams separated from solution, on cooling. These were saponified with alcoholic potassium hydroxide, diluted with water, and extracted with ether to remove unsaponifiable material. The yield of acids was 13.4 grams.

The filtrate containing the soluble methyl esters was diluted with water and extracted with ether. The concentrated extract was saponified, extracted with ether to remove unsaponifiable material, acidified, and the acids recovered. The yield was 5.6 grams of acids.

Analysis of these acid fractions gave the following values:

	Fraction A *Acids from insoluble methyl esters*	*Fraction B* *Acids from soluble methyl esters*
Melting point (without recrystallization)	76° C.	Sticky semi-solid
Mol. Wt. (by saponification)	355	345
Iodine No. (Hanus)	0.17	5.4
Acetyl No.	10.4	......

Fraction A—acids from insoluble methyl esters

Fraction A was esterified with ethanol and fractionally distilled from a Ladenburg flask.

TABLE 1

Data on distillation of esters of Fraction A

Fraction	Pressure	Temperature	M.P. of esters	M. Mol. Wt. of esters	Wt. of fraction
No.	*mm.*	*°C.*	*°C.*		*Grams*
A-1	1.0	165–200	50.0	380.0	4.67
A-2	1.0	200–205	51.2	384.0	7.68
A-residue	....	205–210		397.0	0.75

By repeated recrystallization of the acids of Fraction A-2, 1.5 grams of lignoceric acid[1] was isolated. It had a m.p. of 80° C. and a molecular weight of 368.4 (Theor. 368.38). About 0.2 gram of lignoceric acid was also isolated from the residue. The isolation of this acid from Fraction A-1 and its purification was attained only with great difficulty. The acetyl value indicated the presence of one or more hydroxy acids. Presumably the presence of such acids was the source of the difficulty experienced in purifying the lignoceric acid. The residues were combined, re-esterified and finally redistilled and the acids isolated. Attempts at isolation from this fraction of acids of lower molecular weight than that of lignoceric acid resulted each time only in the final isolation of material consisting entirely or mostly of lignoceric acid.

Assuming that the acids present in the fractions analyzed have an average molecular weight equal to that of stearic acid, a calculation based upon the molecular weight data obtained on each of the fractions indicates that the material of Fraction A consists of approximately 80 per cent of lignoceric acid.

Fraction B—acids from soluble methyl esters

After removal of the bulk of the lignoceric acid from the acids of this fraction by crystallization from ethanol the remaining acids were converted into lead salts and extracted with ether to separate the saturated and unsaturated acids. From the ether-insoluble lead salts 1.0 gram of acids and from the ether-soluble lead salts 0.25 gm. of acids was recovered. The following analytical values were obtained:

	Fraction B-1 *Acids from ether-insoluble lead salts*	*Fraction B-2* *Acids from ether-soluble lead salts*
Mol. wt.	303	369.7
Acetyl value	7.8	19.6
Iodine No. (Hanus)	3.7	28.3
Yield in grams	1.0	0.25

The acids from the ether-insoluble lead salts (Fraction B-1) were fractionated by distillation under reduced pressure with the aid of an electrically heated semi-micro fractionating column. The data are given in table 2.

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[1] Jantzen and Tiedcke (5) found that the lignoceric acid obtained from peanut oil is a mixture. Chibnall, Piper, and Williams (6) found that the lignoceric acid obtained from brain cerebrosides also is a mixture. They thought that its most probable composition is: 10 per cent hexacosanoic, 80 per cent tetracosanoic, and 10 per cent docosanoic acids.

TABLE 2

Data on distillation of acids from ether-insoluble lead salts

Fraction	Pressure	Temperature	M. Mol. Wt. (by Sapn.)	Wt. of fraction
No.	*mm.*	°*C.*		*grams*
1	1.5	–190°	259.6	.35
2	1.5	190–205°	300.7	.13
3	2.0	230–232°	331.3	.20
Residue	......	....	330.7	.20

From the mean molecular weight of the first fraction (259.6) it was evident that one component had a molecular weight at least as low as that of myristic acid. The first as well as the second fraction was subjected to steam distillation and a product was finally isolated which had a molecular weight of 228.8, or that of myristic acid. Other products were isolated from the steam distillates, varying in their mean molecular weight from 240 to 300, but in all cases the analytical values were such as to indicate mixtures. The values in table 2, indicate that at least 50 per cent of the acids in this fraction are of a molecular weight greater than 300.

The acids from the ether-soluble lead salts (Fraction B-2) after treatment with norite were obtained as a light brown viscous liquid. Their mean molecular weight (369.7) is that of an acid of 24 carbon atoms. Because of the extremely small amount of material available, the nature of the acids of this fraction can only be conjectured. The analytical values do, however, indicate that these acids are of types similar to those found from other analogous sources of lipids. The acetyl value indicates a hydroxy acid, and the iodine value an unsaturated acid. In view of the fact that a saturated hydroxy acid of so high a molecular weight would probably form an ether-insoluble lead salt, it is likely that the hydroxy acid of this fraction is also unsaturated. Klenk (7) has isolated such an acid, an α hydroxy tetracosenoic acid, from the cerebrosides of the brain. The iodine number is sufficiently high to account for, in addition, a non-hydroxy unsaturated acid. Nervonic acid, a mono unsaturated acid of 24 carbon atoms is also characteristic of brain cerebrosides.

SUMMARY

The acetone-insoluble, ether-insoluble lipids of milk were isolated and found to consist of approximately equal parts of sphingomyelin and cerebrosides. Their amount was approximately 15 per cent of that of the ether soluble lecithin-cephalin material formerly isolated and analyzed.

Their fat acid constituents were analyzed and found to consist chiefly of lignoceric acid (approximately 80 per cent) which was isolated and iden-

tified. The other 20 per cent of acids was found to be a very complex mixture of saturated, unsaturated, hydroxy saturated, and presumably hydroxy unsaturated acids, no one with the exception of myristic acid being present in sufficiently great concentration to make isolation possible with the limited supply of available material. The indications were that myristic acid is present in a concentration of less than 1 per cent. The presence of lignoceric acid in high concentration and the indicated nature of the other acids are in harmony with the nature of the acids of sphingomyelin and cerebrosides obtained from other sources.

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COOKED AND OXIDIZED FLAVORS OF MILK AS AFFECTED BY FERROUS IRON*

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INTRODUCTION

In a previous study (1) data were presented showing the correlation between cooked and oxidized flavors of milk, the cooked flavor being differentiated from the "pasteurized" or "heated" flavor which frequently results from ordinary pasteurization procedures. A relationship was found to exist between copper-induced oxidized flavor and the cooked flavor when the copper was added before heating the milk, whereas there was no such relationship noted when the copper was added subsequent to heating. In the former case, the cooked flavor usually appeared at temperatures of 84–86° C., whereas temperatures of 76–78° C. sufficed to cause the flavor when the copper was added following the heat treatment. Coincidental with the appearance of the cooked flavor when the copper was added before heating was the failure of the milk to develop the oxidized flavor, *i.e.*, the milk heated to 84–86° C. or above did not become oxidized whereas that heated to lower temperatures developed this flavor defect. Such a relationship between the cooked and oxidized flavors did not exist when the copper was added following the heating and all of the samples became oxidized even though heated to 90° C.

Further, this study showed hydrogen sulphide liberation to be correlated with the cooked flavor and to be responsible for the heat retardation of the development of the oxidized flavor. Copper and ferrous iron both were found to have a retarding influence on the cooked flavor, with the copper being decidedly more effective than iron in this respect. The difference between these metals in this connection was ascribed as being due perhaps to the greater affinity of copper for the sulphides which were formed during the heat treatment. For a fuller explanation of the problem and for a complete literature review the reader is referred to the previous publication (1).

Since the earlier work in this connection dealt chiefly with the relation of cooked and oxidized flavors when copper served as the catalyst for inducing the oxidized flavor, it was thought desirable to ascertain if ferrous iron would give similar results. Consequently, the study reported in this paper was conducted to determine (a) the relation between the cooked and iron-induced oxidized flavors when the iron is added before and after heating the milk, (b) the possible correlation of the findings with the liberation of the milk sulphides.

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EXPERIMENTAL

Procedure: The milk used in this study was from the mixed herd supply of the College creamery. The heating trials were conducted using a 5-liter round bottom flask suspended in a boiling water bath. An electrically driven glass-rod agitator stirred the milk during its heating period. A bent glass tube was used as a siphon by which samples were taken for flavor examinations. An accurate thermometer was suspended in the milk during its heat treatment.

Approximately 3.5 liters of milk were heated in one trial. The milk was raised to the desired temperature in about 15 minutes. The temperatures at which the samples were taken were accurate within ± 0.5° C. The samples were collected in glass sample jars and were immediately cooled by placing the jars in cold water.

Cooked flavor determinations were made within 30 minutes following the heat treatment, and the milk was again examined both for cooked and oxidized flavors after 48 hours storage at 40° F. The samples were keyed in such a manner that their identity remained unknown to the judges. Flavor examinations were made usually by two experienced milk judges.

The oxidized flavor was developed by the addition of a ferrous chloride solution added at a rate of 2.8 p.p.m. of the iron. The iron was added either immediately before or immediately after the milk had been heated.

The sulphide determinations were conducted as described previously (1). In brief, the method consists of heating and aerating 4.5 liters of the milk and drawing the vapors through a filter paper dampened with lead acetate solution. The darkening of the paper due to the formation of lead sulphide indicates the liberation of hydrogen sulphide from the milk.

Results: Results secured when the iron-contaminated milk was heated are illustrated in figure 1. The figure represents the results from five different

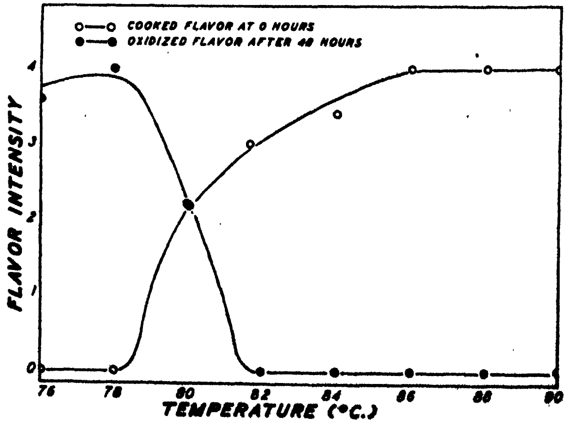

FIG. 1. The cooked and oxidized flavors of milk to which was added 2.8 p.p.m. of ferrous iron before heating.

trials, all of which gave practically identical results. The intensity of the flavor was graded as follows: 1 = questionable, 2 = faint, 3 = distinct, and 4 = strong.

The graph shows a definite relation between the cooked and oxidized flavors. Temperatures of 80–82° C. or higher usually produced the cooked flavor, whereas the lower temperatures did not. These cooked flavor results were in general unchanged whether the flavor examination was made immediately after heating or after the 48-hour storage period. This is a point of difference between these findings and those reported earlier for copper (1). The oxidized flavor usually developed in those samples which were heated to 80° C. or lower, whereas the flavor failed to develop in those samples subjected to higher temperatures. In other words, samples which possessed the cooked flavor did not become oxidized, and vice versa. Exceptions occurred in the milk heated to 80° C. which at times was criticized as being cooked and also developing an oxidized flavor. This temperature, *i.e.*, 80° C., might be said to be the critical temperature in this connection and is the dividing point between the oxidized and cooked flavors. These results are similar to those reported earlier for copper with the exception that the critical temperature for copper was found to be approximately 84–86° C.

Several trials were also conducted to determine the effect on the cooked and oxidized flavors of milk of adding the ferrous iron after heating. These results are portrayed in figure 2, and in general are not greatly different from those illustrated by figure 1. This graph, however, does show the influence of the storage period upon the cooked flavor. The cooked flavor of the milk, as determined immediately after the heat treatment, occurred at the normal temperature of 76–78° C. Following the addition of the iron

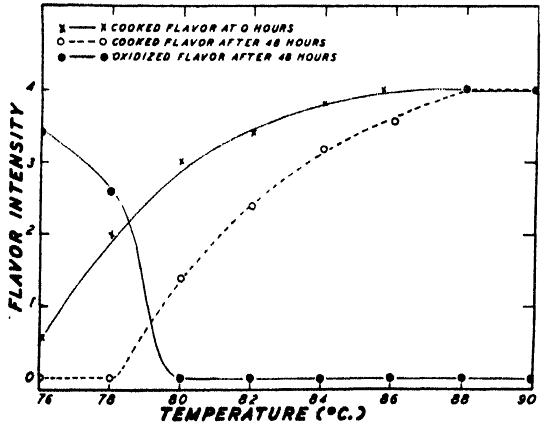

FIG. 2. The cooked and oxidized flavors of milk to which was added 2.8 p.p.m. of ferrous iron after heating.

and after the 48-hour period, the lowest temperature at which the cooked flavor was detected was 80° C. As in the case when the iron was added before heating approximately 80° C. was found to be the critical temperature. However, there seemed to be a slight tendency for this temperature to be shifted to a lower point although the difference appears negligible. The graph shows that the milk heated to 76° or 78° C. developed an oxidized flavor, whereas this flavor did not develop in the milk heated to 80° C. or above. Conversely, the cooked flavor was present in the milk heated to 80° C. or above even after 48 hours of storage. The cooked flavor observed immediately after heating in those samples heated to 76° or 78° C. had disappeared during storage, being replaced by the oxidized flavor.

Figures 1 and 2 show that the influence of heat in preventing the development of the oxidized flavor as induced by ferrous iron is equally as effective irrespective as to whether the iron was added before or after heating. This indicates a difference in the influence of heat on the oxidized flavor caused by iron or copper, inasmuch as the previous study (1) showed the copper-induced flavor to be greatly affected by the period of contamination, viz., whether before or after heating. Further, the fact that the cooked flavor of the milk persisted following storage irrespective as to whether the iron was added before or after the heating process, indicates another difference between the action of iron and copper in regard to this flavor.

To verify the difference between copper and iron in affecting the disappearance of the cooked flavor, samples of milk were heated to various temperatures and following cooling were divided into three lots. Lot 1 served as the control, Lot 2 contained 2.8 p.p.m. of added ferrous iron, and Lot 3 contained 1.4 p.p.m. of added copper. The samples were stored at approximately 4° C. and were examined for cooked flavors after 0, 6, 24, and 48 hours. The results are presented in table 1, and represent the average of five trials.

These data show the ferrous iron to have no appreciable effect on the persistence of the cooked flavor when the milk had been heated to 82° C. or above. Generally, the cooked flavors of these samples were equally as intense as in the control even after the 48-hour period. However, when the milk had been heated to lower temperatures, the iron-contaminated samples showed more rapid disappearance of the cooked flavor than was observed in the control, although the difference was not great. The samples containing iron which had been heated to 76°–78° C. usually showed oxidized rather than cooked flavors following storage.

On the other hand, the copper had a marked effect on the persistence of the cooked flavor and brought about its total disappearance in all samples by the end of 48 hours. In fact, the flavor had usually disappeared by the end of the 24-hour period, and was usually present after 6 hours only in

TABLE 1

The persistence of the cooked flavor of milk as influenced by the addition of copper or ferrous iron after heating

Temperature of heating	Storage period (hours)											
	0			6			24			48		
	Lot number*			Lot number			Lot number			Lot number		
	1	2	3	1	2	3	1	2	3	1	2	3
°*C.*												
76	1.2	0.0	0.2	1.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
78	3.2	2.8	1.2	3.2	2.3	0.0	2.4	2.2	0.0	0.6	1.0	0.0
80	4.0	3.6	2.4	4.0	3.3	0.0	4.0	3.2	0.0	3.4	2.8	0.0
82	4.0	3.8	3.4	4.0	3.6	0.0	4.0	3.8	0.4	3.8	4.0	0.0
84	4.0	4.0	3.6	4.0	3.8	0.0	4.0	3.8	0.8	3.8	4.0	0.0
86	4.0	4.0	3.8	4.0	4.0	0.8	4.0	4.0	0.8	4.0	4.0	0.0
88	4.0	4.0	3.6	4.0	4.0	2.0	4.0	4.0	1.2	4.0	4.0	0.0
90	4.0	4.0	3.8	4.0	4.0	2.8	4.0	4.0	1.2	4.0	4.0	0.0

* Lot 1 is the control. Lot 2 contains 2.8 p.p.m. added iron, and Lot 3 contains 1.4 p.p.m. added copper. In order to average the results the flavors were scored as follows: 0 = no flavor, 1 = questionable, 2 = faint, 3 = distinct, 4 = strong.

those samples heated to 88° and 90° C. The copper-containing samples were all oxidized by the close of the storage period.

The influence of copper and iron on the persistence of the cooked flavor of milk heated to 80° C. is illustrated by figure 3. This graph shows the marked acceleration of the disappearance of the cooked flavor when copper is added to the milk following the heat treatment, and shows further that ferrous iron has only a small and rather insignificant effect in this connection.

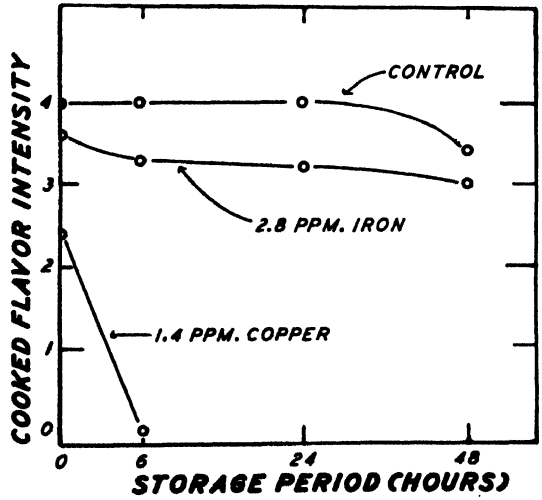

FIG. 3. The influence of copper and iron on the cooked flavor when added to milk which had been heated to 80° C.

The ability of these metals, *i.e.*, ferrous iron and copper, to affect the liberation of hydrogen sulphide from milk may be correlated directly with their ability to affect the cooked flavor. The influence of these metals on the liberation of the heat labile sulphides of milk as detected by lead acetate paper is shown in table 2.

TABLE 2

*The influence of ferrous iron and copper on the heat labile sulphides of milk**

Treatment of samples	Temperature of heating (°C.)							
	76	78	80	82	84	92	94	98
	Intensity of discoloration of lead acetate paper							
Trial 1								
Milk	0	+	++	–	–	–	–	–
Milk and Fe(ous)	–	0	+	+	++	–	–	–
Milk and Cu	–	–	–	–	–	–	0	0
Trial 2								
Milk	0	0	+	++	–	–	–	–
Milk and Fe(ous)	0	0	+	++	–	–	–	–
Milk and Cu	–	–	–	–	–	0	+	–
Trial 3								
Milk	0	+	++	–	–	–	–	–
Milk and Fe(ous)	0	0	+	++	–	–	–	–
Milk and Cu	–	–	–	–	–	0	0	0

* Iron added at the rate of 2.8 p.p.m. and copper at the rate of 1.4 p.p.m. before heating. Discoloration of lead acetate paper graded as follows: 0 = no discoloration, + = faint discoloration, ++ = distinct coloration (– represents no sample).

These results show that the iron has little retarding influence on the liberation of the sulphides with both the normal milk and the iron-treated milk giving a visible discoloration of the lead acetate paper at temperatures of 78° to 80° C. The normal milk tended to show the discoloration at a slightly lower temperature than the iron containing samples, which is to be expected on the basis of the relationship of the sulphide liberation to the cooked flavor. The copper had a marked influence on the sulphide liberation with no sulphide having been detected in two of the trials even though the milk was heated to 98° C. In one of the trials slight discoloration of the lead acetate disc was noted at 94° C.

DISCUSSION

The data reported herein verify those previously reported (1). The influence of heat on retarding iron-induced oxidized flavor is even more pronounced than in the case of copper, due probably to differences in the combining properties of these metals, either with the liberated sulphide or with the substance furnishing this sulphide, or to differences in oxidizing intensities of the two metals. The almost total lack of combining or oxidizing power by the ferrous iron is reflected by the fact that the oxidized flavor results were practically the same whether the iron was added before or after the heat treatment. This is further emphasized by the change in the cooked flavors after varying periods of storage.

The close relationship between the cooked and oxidized flavors of milk, and the correspondingly close correlation between the cooked flavor and sulphide liberation, serves to offer further proof that the reducing system which is formed when milk is heated is responsible for the retardation or prevention of oxidized flavor development, rather than that some enzyme is inactivated.

On the basis of these studies, temperatures of 80° C. or above must be used to prevent the oxidation processes which are induced in milk by ferrous iron. Apparently, lower temperatures are not sufficient to create a reducing system capable of hindering these oxidative changes, at least when the iron concentrations are in the range of 1.4 to 2.8 p.p.m.

CONCLUSIONS

The heating of milk momentarily to 80°–82° C. or above prevents the development of an oxidized flavor as induced by 2.8 p.p.m. of ferrous iron. This occurs irrespective as to whether the iron is added before or after the heat treatment.

The appearance of a strong cooked flavor at these temperatures is correlated with the failure of the oxidized flavor to develop. The liberation of sulphides is related to the cooked flavor and prevents the development of the oxidized flavor.

The "critical" temperature is different for milk containing added ferrous iron than for that containing added copper, due to differences in the sulphide combining abilities of these metals, due to differences in their abilities to form complexes with the substance supplying this heat labile sulphur, or due to differences in their oxidizing properties.

ACKNOWLEDGMENTS

The author expresses his appreciation to Professor G. M. Trout, Michigan State College, for his assistance in judging the milk samples.

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EXPECTATION OF LIFE IN DAIRY COWS*

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Although the expectation of life in humans has been widely investigated and the information obtained about it extensively used by insurance companies and other agencies, there is little information relative to the expected span of life in dairy cattle or other farm animals. Such information on dairy cattle would be useful to the breeder, the buyer and the experimenter, who could then gauge their programs and objectives on something more substantial than guesses.

It is known that replacement rates in dairy herds are rather high, for investigations (1, 2, 5) show it is necessary to replace approximately 21 to 28 per cent of the milking herd annually. Although there is a relationship between replacement rates and expectation of life in dairy cows, these replacement rates yield no accurate information on the expected life span of cows of different ages.

Lush and Lacy (1) and A. D. B. Smith (4) have each made life expectancy tables for dairy cattle. Lush and Lacy's table represents the condition found in registered herds as their table was estimated from data taken from herd books of each of the five major dairy breeds used in the United States, while Smith's table is based on a study of 2600 Scottish cows. We undertook our study to supplement and enlarge what is already known and also to construct a table that would be representative of dairy animals in Iowa and the midwest, when kept under cow-testing-association conditions.

DATA USED

It is customary in Iowa cow-testing associations to keep records on all cows in the herd with the exception of (a) permanent nurse cows,* (b) valuable purebred cows kept for breeding purposes and not milking at any time during the testing association year, and (c) heifers freshening too young and dried up at once or heifers aborting and dried up immediately.

Therefore, the available records included every cow that had entered the milking herd in any year, regardless of the length of time it remained in the herd.

These records were available for the year 1927–28, for the years 1930–35 and for 22 of the 52 associations operating in the year 1935–36. They

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* A permanent nurse cow is one that nurses a calf throughout the entire lactation period. Cows milked at any time during the lactation period are not considered as permanent nurse cows and are included in the records.

TABLE 1

Age distribution of all cows in Iowa cow-testing associations for years 1927–28 and 1930–36 regardless of the length of time they remained in the herd

Year	Age in years														No record	Total
	2–3†	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15 and over		
1927–28	2226	1949	1372	1134	1019	875	742	395	312	179	130	71	36	35	1027	11502
1930–31	8469	5906	4829	4113	3317	2568	1929	1214	900	459	390	150	98	90	4725	39157
1931–32	6514	4446	3529	3047	2655	1844	1418	927	644	382	248	139	84	72	3335	29284
1932–33	4411	3259	2511	1957	1711	1296	966	713	436	247	204	76	69	57	2318	20231
1933–34	4298	3089	2572	1925	1570	1220	1014	608	444	245	163	94	43	39	3177	20501
1934–35	3877	2962	2394	1888	1580	1140	910	649	416	238	175	69	44	32	2286	18660
1935–36*	1652	1124	1051	776	683	528	382	270	186	79	52	36	21	13	1408	8261
Total	31447	22735	18258	14840	12535	9471	7361	4776	3338	1829	1362	635	395	338	18276	147596

* Cows from only 22 of the 52 associations in operation during 1935–36 are included, as the records from the rest of the associations were not available.

† The figures in this column have been corrected by using 82.92 per cent of the original count in order to eliminate an error caused through cows having been recorded twice as two year olds on consecutive years. A sample test covering 6538 two year old animals showed that 17.08 per cent of them were recorded twice.

covered both purebred and grade cows; the number of purebreds varied upwards from approximately 20 per cent of the total for 1927–28 to 34 per cent for the most recent year.

All cows of each herd were sorted from these records on the basis of age. The summary of this sorting is shown in table 1 which gives the total number of animals of each age for each testing-association year. One column also records the number of cows whose ages were unknown.

Having the number of cows of each age, it is assumed that the difference in numbers represents the mortality of cows as they progress from one age to the next one higher. By mortality of cows, we do not mean that all the cows actually died, but we mean that the cows were removed from the milking herds for one cause or another, such as culling for low production, disease, old age, etc.

By applying the formula $e_x = \frac{T_x}{l_x}$, given by Pearl (3) to the figures representing the total number of cows for each age, the expectation of life for this group of cows was calculated. This formula was used because the cows in each age group were distributed so that it contained cows barely old enough to get into it as well as cows just too young to enter the age group next older. For purposes of comparison, we also calculated the expectation of life for the cows that had passed through the Iowa State College herd during its existence. Table 2 shows the expectations of life for both the cow-testing-association cows and the college cows. The data from the cow-testing associations grouped all cows 15 years and older together. Because some of these cows may have lived to be 18, 19 or more years of age, a discrepancy entered our calculations which was rather large for the older ages, but tended to rapidly lose its force with the younger ages. For that reason, we have extended this table only to cows with ages of 12–13 years.

TABLE 2

Expectations of life of cows of different ages in Iowa cow-testing associations and in the Iowa State College dairy herd

Age in years	Expectations of life in years	
	C.T.A. cows	College cows
2– 3	4.1	4.2
3– 4	4.3	4.0
4– 5	4.1	3.8
5– 6	3.8	3.7
6– 7	3.4	3.3
7– 8	3.1	3.1
8– 9	2.7	2.9
9–10	2.7	2.4
10–11	2.4	2.4
11–12	2.5	2.2
12–13	2.0	2.2

With one or two exceptions, the life expectancies for the two groups are similar. Perhaps the greatest differences lie in the expectancies for 2–3- and 3–4-year-olds, where the expectancy for the 2–3-year-olds in the cow-testing-association group was less than for the 3–4-year-olds. The extent of the differences is more clearly seen in table 3 which shows the number of cows of each group that would be left in the herd at each age for each hundred 2–3-year-olds which entered the herd, providing animals left the herd in the ratios shown by the age distributions of the two groups.

TABLE 3

Number of cows that would be left in the herd at each age for each original hundred 2–3-year-olds providing the animals left the herd in the ratio shown by the age distribution of each group

Age in years	Number of animals at each age	
	C.T.A. herds	College herds
2– 3	100.0	100.0
3– 4	72.3	77.1
4– 5	58.1	57.4
5– 6	47.2	41.7
6– 7	39.9	31.7
7– 8	30.1	22.5
8– 9	23.4	14.8
9–10	15.2	11.2
10–11	10.6	5.2
11–12	5.8	2.8
12–13	4.3	1.2
13–14	2.0	0.8
14–15	1.3	
15 & over	1.1	

There were some differences in selecting and sorting the cows in these two groups. Only cows that had freshened were used in the cow-testing-association figures and whenever a cow first freshened, she was called a 2–3-year-old, even though she might not have reached quite the age of two years.

After the counts were completed, it was discovered that some of the animals, probably yearlings and the two-year-olds that calved late in the cow-testing-association year, were entered on the reports as two-year-olds in two consecutive years which increased the counted 2–3-year-olds by that amount. To eliminate this error, a random sample of 6538 two-year-olds was taken from the source data and checked. It was determined that 17.08 per cent of this number had been counted twice on consecutive years. Assuming that this percentage of recounts held true for all the 2–3-year-olds, the original count was reduced by this amount and the corrected figure used in all our calculations.

In sorting the college cows, all two-year-olds were counted whether they had freshened or not, while no yearlings were included, even though they had freshened.

The differences that existed in the selecting and sorting of the cows in the two groups do not account entirely for the 2–3-year-olds of the cow-testing-association group having a shorter life expectancy than the 3–4-year-olds of this same group, while the reverse is true of the college cows. A larger proportion of 2–3-year-olds left the cow-testing-association herds than was the case with the college herd. In the college herd, the recorded reasons for disposal indicate that with few exceptions cows left this herd largely because of disease, bad udders such as the loss of a quarter, and sterility. As would be anticipated under these circumstances, the expectation of life for the 2–3-year-olds is greater than for 3–4-year-olds. If the college herd expectation of life relationship for 2–3- and 3–4-year-olds is the correct one, where little culling other than "forced" culling takes place, then it is apparent that other kinds of culling, such as for low production, have taken place in the cow-testing-association herds. The amount of this culling can be estimated from table 3 for in the college herd, 23 out of each 100 2–3-year-olds were eliminated, while in the cow-testing-association herds, 28 out of each 100 2–3-year-olds left the herds or 5 more than were culled from the college herd. This would indicate that at least 5 per cent of the 2–3-year-olds of the cow-testing-association herds were voluntarily culled by owners.

Some of the years, particularly 1932–35, were affected by the business depression and also drought which caused a shortage of feed. These conditions might have altered the rate and age of culling, but from table 4 it can

TABLE 4

Percentages of 2–3-year-old cows that remained in the cow-testing-association herds to enter each successive year

Year	Age of cows in years													
	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15 and over
1927–28	100.0	87.6	61.6	50.9	45.8	39.3	33.3	17.7	14.0	8.0	5.8	3.2	1.6	1.6
1930–31	100.0	69.7	57.0	48.6	39.2	30.3	22.8	14.3	10.6	5.4	4.6	1.8	1.2	1.1
1931–32	100.0	68.3	54.2	46.8	40.8	28.3	21.8	14.2	9.9	5.9	4.4	2.1	1.3	1.1
1932–33	100.0	73.9	56.9	44.4	38.8	29.4	21.9	16.2	9.9	5.6	4.6	1.7	1.6	1.3
1933–34	100.0	71.9	59.8	44.8	36.5	28.4	23.6	14.1	10.3	5.7	3.8	2.2	1.0	0.9
1934–35	100.0	76.4	61.7	48.7	40.8	29.4	23.5	16.7	10.7	6.1	4.5	1.8	1.1	0.8
1935–36	100.0	68.0	63.6	47.0	41.3	32.0	23.1	16.3	11.3	4.8	3.1	2.2	1.3	0.8
All cows	100.0	72.3	58.1	47.2	39.9	30.1	23.4	15.2	10.6	5.8	4.3	2.0	1.3	1.1

be seen that except for one year (1927–28) the rate of culling was somewhat the same in all years.

The formula used to calculate the expectations of life applies to populations that are static. It is assumed that replacements within these herds were made through the addition of animals 2–3 years of age or younger. It is recognized that this assumption may not be true as a majority of the herds may have either been increasing or decreasing during the period of the study. Probably some were increasing in size while others were decreasing, so that one would cancel out the other, thus tending to maintain the total number relatively static.

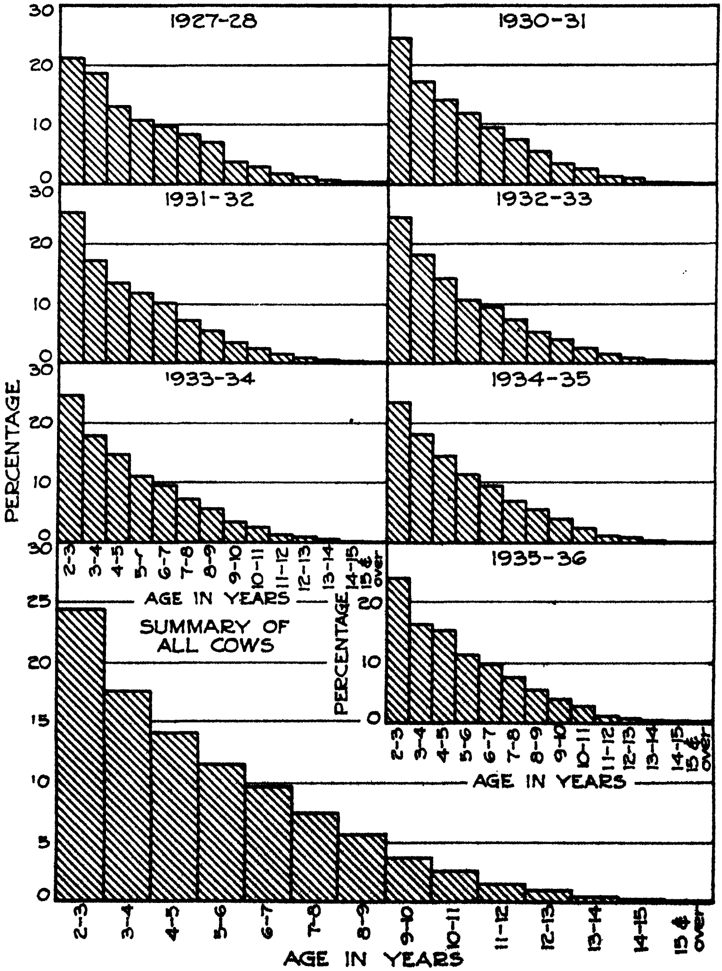

FIG. 1. Percentage distribution by ages of cow-testing-association cows for each year studied and for all cows.

It could happen that instead of the replacements being brought into the herds at 2–3 years of age or younger, that some were purchased at an older age. If this happened, the calculated expectations of life would be changed more as the numbers of such cows increased. Since most of Iowa's dairymen are dairying in order to market the crops from their farms, the replacements for their herds are usually raised on their own farms, tending to keep movement of older cows into herds at a minimum. When purchases are made, they may come from other members of cow-testing associations, in which case there would be no influence on the results.

The expectations of life which were obtained in this study were shorter than those obtained by Lush and Lacy (1) but this can be accounted for partially through the differences in the populations studied. In both the cow-testing-association population and that of the Iowa State College herd, all animals which entered the milking herd were counted, regardless of the length of time they remained in the herd and whether they later calved or not. On the other hand, the populations studied by Lush and Lacy (1) were selected only because they had produced a living calf which was recorded in one of the major dairy breed's herd books. Their data show a tendency for the dams of bulls to be older (2–6 months) than the dams of females. If, in addition, there was a tendency for breeders not to register females from young dams, it would create a bias in the "life expectancies," probably lengthening them.

Frequently, one wishes to know the percentage of cows of each age that make up an average herd. Table 5 gives the percentage distribution by ages of all cows in the cow-testing-association herds used in this study and figure 1 shows not only the percentage distribution by ages of all the cow-testing-association cows for all years but also their distribution for each year.

The table shows that about 56 per cent of all animals in the cow-testing-association herds were under 5 years of age. A rough calculation of the

TABLE 5

Showing percentage of all cows in cow-testing-association herds that are in each age group

Age in years	Percentage
2– 3	24.31
3– 4	17.58
4– 5	14.11
5– 6	11.47
6– 7	9.69
7– 8	7.32
8– 9	5.69
9–10	3.69
10–11	2.58
11–12	1.41
12–13	1.05
13–14	0.49
14–15	0.31
15 or over	0.26

average age of all cows in this group showed the age to be about 4.7 years. This figure is probably slightly low, as in making this calculation, all cows in the group "15 years or over" were given the arbitrary age of 15 years, even though some of the cows were older than this. For comparison, the average age of cows in the college herd was calculated and found to be 4.2 years.

SUMMARY

Iowa cow-testing-association records for seven years yielded information about the ages of 147,596 cows, from which was constructed a table of expectation of life. These expectancies of life were compared with others which were based on the age histories of cows in the Iowa State College herd. Variations existed in these two tables in the life expectancies of 2–3-year-olds and 3–4-year-olds. The 2–3-year-olds of the cow-testing-association group had shorter and the 3–4-year-olds longer life expectancies than these same age groups in the college herd. At other ages, the expectations of life were similar in their relationships.

The low expectation of life for the 2–3-year-olds of the cow-testing-association group was probably due to more culling in these herds at this age than took place in the college herd.

A rough calculation showed the average ages of the cows in cow-testing-association herds to be approximately 4.7 years, and those in the Iowa State College herd to be 4.2 years.

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ERRATA

April, 1939, page 218, reference (1) should read: Barthel, Chr., Weitere Untersuchungen über die Reduktaseprobe, sowie Vergleiche mit einigen anderen neueren milchhygienischen Untersuchungsmethoden. Zschr. Untersuch. Nahrungsmitt. 34: 137–164. 1917.

April, 1939, page 248, reference (16) should read: Fürth, O. von, and Charnass, D., Über die quantitative Bestimmung der Milchsäure durch Ermittlung der daraus abspaltbaren Aldehydmenge. Biochem. Zschr. 26: 199. 1910.

April, 1939, page 249, reference (33) should read: Scheunert, A., and Pelchrzim, H. von, Über den Gehalt des Blutes verschiedener Tierarten an Zucker, Rest-N, Harnstoff-N, Kreatininkörpern und Harnsäure nach den Folinschen Methoden. Biochem. Zschr. 139: 17–29. 1923.

April, 1939, page 249, reference (45) should read: Abderhalden, E., Zur quantitativen vergleichenden Analyse des Blutes. Z. Physiol. Chem. 25: 65. 1898.

June, 1939, page 436, line 25 should read: The proteins of corn gluten meal do not appear to entirely make good the cystine deficiencies of alfalfa crude protein.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

1. Detecting Thermophilic Contamination in Skim-milk Powder.

C. M. SORENSEN, Sealtest Research Laboratories, Baltimore, Md. Food Research, *3*, 4, p. 421. July, Aug., 1938.

A method for determining contamination of dry skimmilk with organisms of the "flat-sour" type, utilizing N/60 sodium or lithium hydroxide for solvation of the dry milk, dextrose-tryptone brom cresol-purple agar for plating and an incubation temperature of 55° C., is described. F.J.D.

CHEESE

2. Methods for Determining Salt in Various Cheeses.

J. C. MARQUARDT, N. Y. Agr. Exp. Station, Tech. Bull. No. 249, Sept., 1938.

This publication represents a thorough study of procedures for determining the salt content of several varieties of cheese. It contains a literature review that starts with the original work on this subject by J. Volhard in 1878. A complete review of faulty procedures and the significant developments to produce proper procedures is given.

The study has been undertaken because of previous findings at the station which stress the importance of proper salt contents in cheeses.

The modified Volhard method, approved by the American Dairy Science Association, gives reliable results with young cheddar, aged cheddar, roquefort, blue vein, camembert, limburger and cottage cheeses. Other varieties were not studied.

It was possible to obtain reliable results by titrating directly aliquots of solutions prepared from freshly made cheeses less than five days old. Potassium chromate and dichloro-fluorescein gave comparable results when used as indicators for the direct titrations. Two methods for the direct titration have been described.

It is not possible to obtain accurate values with cured cheeses by direct titration.

Water soluble proteins render direct titrations unsuitable for certain cheeses over 5 days old. A study has been made of the influence of time of solution, temperature of water, reaction of the aliquot and like factors in regard to their influences upon direct titrations. J.C.M.

3. Warm Water Washing Improves Sweet Curd Cottage Cheese.

L. J. MANUS, Department of Dairy Husbandry, West Virginia University. Milk Dealer, *27*, 12, pp. 40, 70–71, September, 1938.

Washing of soft, properly coagulated curd flakes with warm water was found to result in the production of curd that was not only soft but sweet in taste. The detailed procedure, as used successfully at the West Virginia Creamery for more than two years, is given. C.J.B.

DISEASE

4. Post Parturient Hemoglobinuria of Cattle. FARQUHARSON, J. AND SMITH, K. W., Colorado State College, Fort Collins, Colorado. Journal of the American Vet. Med. Ass'n. Vol. XCIII—N. S. Vol. 46—No. 1, pp. 37–39, July, 1938.

Referring to this post parturient disease of well nourished mature cows, the authors state that the malady is characterized by its sudden onset and voiding of blood-colored urine, and its appearance principally in winter and spring months. Symptoms may be confused, by casual observation with those of milk fever. The causes of the malady are unknown at present. *Treatment*—place affected cow in clean, quiet surroundings—remove concentrates from ration and give only alfalfa hay of good quality. Use blood transfusion (direct) after signs of weakness develop and repeat as often as maintenance of strength requires. Recovery is slow. *Prevention*—Use dietary measures around time of parturition—remove concentrates from ration, three weeks before and two weeks after parturition and feed only good quality alfalfa hay. The authors warn that this is not a *definite* means of prevention. J.W.W.

5. The "Saturation Method" for the Treatment of Breeding Failures with Wheat-Germ Oil. PACINI, AUGUST J., Minneapolis, Minnesota. J. Am. Vet. Med. Assn. Vol. XCIII—N. S. Vol. 46, No. 4,—pp. 236–238, Oct., 1938.

The author believes that the value of wheat-germ oil may be due to ingredients other than Vitamin E and that the therapeutic use of wheat-germ oil cannot be considered synonomous with Vitamin E therapy. In the saturation method one single original dose of four ounces of wheat-germ oil (not obtained by any solvent process) is administered. Every third day thereafter the animal is given a tablespoonful of wheat-germ oil until the time covered is equivalent to one estrous cycle (21 days). Thereafter the animal is furnished grain feed to which four ounces of wheat-germ oil has been added to each ton of grain feed. Careful observations will show a perceptible improvement in the coat of the animal in from two to three weeks after the initial dose. Generally speaking, animals whose coats fail to display an improved condition will fail to respond favorably in the correction of the breeding disorder. J.W.W.

FEEDS AND FEEDING

6. Feeding Value of Hay Crop Silage. C. F. MONROE, C. C. HAYDEN, A. E. PERKINS, W. E. KRAUSS, C. E. KNOOP, AND R. G. WASHBURN, Ohio Agr. Exp. Sta., Wooster, O. Ohio Exp. Sta., Bimonthly Bull. Vol. XXIII, No. 194, Sept.–Oct., 1938.

Three feeding trials were conducted in which a combination of soybean and corn silage was compared with legume hay and corn silage. Two comparisons were made between acid silage (A.I.V.) made from alfalfa and clover mixtures, and ordinary hay rations.

It was shown that hay crop silages may be satisfactorily fed to replace sun-cured hay and that such feeding may be continued for the winter feeding period without apparent ill effect to the cows.

Alfalfa molasses silage was found to be a valuable supplement for cows on pasture, with indications that under certain conditions it may be more valuable than grain in maintaining production. W.E.K.

FOOD VALUE OF DAIRY PRODUCTS

7. The Place of Milk in Nutrition. C. A. ELVEHJEM, Dept. of Biochemistry, University of Wisconsin. Milk Dealer, *28*: 1, pp. 56–62, October, 1938.

The author shows that while milk is the nearest approach we possess to a complete food, it is deficient in iron, copper, and manganese. When these minerals were added to milk and fed to young rats, the animals grew as well or better than on any other mixture of foodstuffs.

By using milk mineralized with iron, copper, and manganese it was demonstrated that milk produced during September and November gave growth entirely comparable with that obtained on a mixed diet. Milk produced in February and March was much inferior and milk produced during December and January was intermediate in its growth-promoting property. Summer evaporated milk gave a daily gain of 3.6 gm., while winter evaporated milk gave a gain of only 1.7 gm. daily. By various supplements to the winter ration the nutritive value of winter milk was improved.

Results for the pasteurized milk showed that the growth on pasteurized summer milk was identical with that on raw summer milk. However, the gain in weight for pasteurized winter milk was inferior to that for raw winter milk. Pasteurization, therefore, has no marked detrimental effect, as measured with rats, upon the nutritive value of a milk of high nutritive quality, but may further reduce the value of a milk of low nutritive quality.

In addition to the reports on winter and summer milk and the effect of pasteurization, it is reported that lactose cannot be utilized except when fed with definite amounts of fat. If the fat is absent the sugar will be lost unchanged in the urine. Thus it is necessary that milk be used with its normal fat content. C.J.B.

ICE CREAM

8. Ice Cream and Cakes, Pies, Cookies. ANONYMOUS, Ice Cream Field *32*: 8, pp. 33, 34, 48, August, 1938.

Some of the advantages of selling ice cream and pastry products at the same time are illustrated by specific examples from the industry. Examples are also given to show that difficulties may arise in such an attempted combination. W.C.C.

9. Ice Cream Specialties. KEN FORREST, Ice Cream Field *32*: 9, pp. 20, 21, 22, 37, September, 1938.

Examples of a variety of different types of ice cream packages which have been successfully merchandized are cited.

It is claimed that with a quality product plus a novelty appeal, the 5¢ item can be responsible for a substantial part of the ice cream manufacturers' profits. W.C.C.

10. Autumn Specials. RALPH BARRETT, Ice Cream Field *32*: 9, pp. 7–8, Sept., 1938.

The author recommonds the following basic procedure as a means of successfully advertising ice cream.

1. Hire a competent home economics specialist to draw up fool proof "recipes" involving the use of commercial ice cream in home made desserts.
2. Prepare your advertising campaign on the basis of the amount of money allotted for this purpose.
3. Continue this program even though the results obtained may seem slow in materalizing.

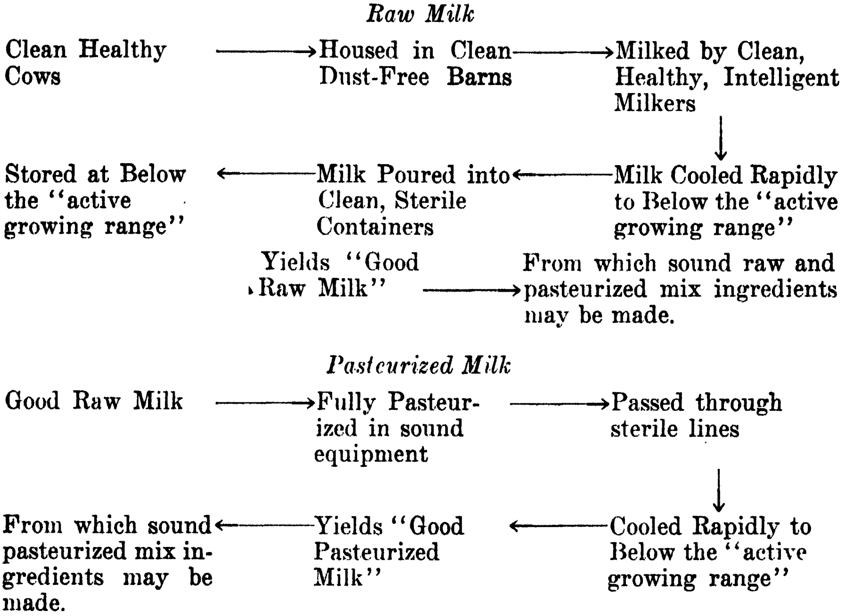
The author lists 14 different combinations of ice cream with other products which he claims have been successfully used in advertising programs based on the above outline. W.C.C.

11. Milk as an Ingredient. DAVIS LEVOWITZ, New Jersey Dairy Laboratories. Ice Cream Field *32*: 7, pp. 23–24. July, 1938.

The author's discussion of this topic can be summarized by the following taken from the article:

Flow Sheet for Good Milk

The Fundamental Consideration for Good Ice Cream Ingredients



Raw Milk

Clean Healthy Cows → Housed in Clean Dust-Free Barns → Milked by Clean, Healthy, Intelligent Milkers ↓

Stored at Below the "active growing range" ← Milk Poured into Clean, Sterile Containers ← Milk Cooled Rapidly to Below the "active growing range"

Yields "Good Raw Milk" → From which sound raw and pasteurized mix ingredients may be made.

Pasteurized Milk

Good Raw Milk → Fully Pasteurized in sound equipment → Passed through sterile lines ↓

From which sound pasteurized mix ingredients may be made. ← Yields "Good Pasteurized Milk" ← Cooled Rapidly to Below the "active growing range"

W.C.C.

12. **Frosted Foods.** M. A. Corbett, New York Group, National Food Distributors Assn., Ice Cream Field *32*: 10, pp. 23, 23, 25, 34, Sept., 1938, 32 p. (11).

The author outlines briefly the early developments of the frosted food industry.

He points out that whereas the early growth was very rapid in many instances that its success will depend upon proper cultivation. He states "competition of the right sort is the thing the industry needs most to give quick freezing a solid footing in the industry."

According to the author many of the large wholesalers of frosted foods have quit the field. He states that confusion in the wholesale and retail branches of this industry is due to the fact that many distributors "are more interested in loading warehouse and store cabinets with merchandise than they are in cooperating to build a permanent, mutually profitable business."

W.C.C.

13. **Advantages of Dry Egg Yolk.** **Walter Halpert**, Stein, Hall & Co., Inc., New York. Ice Cream Field *32*: 7, pp. 16–17, 44. July, 1938.

14. Sanitation and Control. C. W. England, Department of Animal and Dairy Husbandry, University of Maryland. Ice Cream Field *32*: 7, pp. 31–32, 42, July, 1938; *ibid.*, 8, pp. 42–47, Aug., 1938.

According to the author ice cream sanitation includes three aims:

1) It should be clean and free from all extraneous matter. 2) It should be safe, that is, it should not contain disease-producing organisms. 3) It should not contain large numbers of miscellaneous bacteria.

He considers the following sources of contamination:

1) equipment. 2) plant operator. 3) ingredients added to mix after pasteurization. 4) retail dispensing of ice cream. Usually the personal factor is the most difficult to control.

The author discusses the more common ways that high bacteria counts are obtained in ice cream, citing work previously published to confirm most of his statements.

He points out that although high bacteria counts in colors and flavors will not ordinarily increase materially the final count of the ice cream, yet such contaminations should be guarded against.

He claims that the greatest sanitary problem of the counter freezer manufacturer so far as equipment is concerned is the freezer itself. Detailed directions for washing and "sterilizing" it are given. Where steam and hot water are not available chlorine is the only method left. This method leaves the equipment wet and is therefore objectionable. He strongly condemns the use of a cloth to dry the freezer.

He points out that often the high bacteria counts in ice cream served to the public are due to contaminations from the vendor's scoop. Figures taken from the American Journal of Public Health, vol. 27, 1007–9, 1937, show the importance of properly caring for the scoops.

The following is a summary by the author of the main points in producing and dispensing ice cream of excellent sanitary quality.

1) Use raw materials of an excellent sanitary quality and pasteurize at 160° F. or higher for 30 minutes, or if you purchase a pasteurized mix, insist that it be of an excellent sanitary quality and properly pasteurized.

2) Keep the mix below 40° F. and for a period not longer than two days until frozen.

3) Avoid contamination of opened fruits, fruit concentrates, nuts, extracts and colors. Keep these items covered and refrigerated.

4) Properly clean and sterilize the freezer and other equipment with which the mix or ice cream comes in contact.

5) Keep scoops on a dry rack and rinse in running water before and after use.

6) Use common sense coupled with cleanliness. W.C.C.

15. Frosted Foods for Ice Cream Manufacturers. C. Q. SHERMAN, C. Q. Sherman Co., N. Y. Ice Cream Field *32*: 8, pp. 12–13, 22, August, 1938.

The author claims that frosted foods can be handled by ice cream manufacturers to advantage because they already have hardening room facilities that can be used to store frosted foods. In addition delivery trucks used for ice cream can also be used to deliver frosted foods.

The author also states that the dairy routes are potential outlets for frosted foods, but it is to the advantage of the distributor to handle established brands rather than try to sell his own brand of frosted foods.

He claims that in his experience health authorities have not objected to keeping packaged ice cream and packaged frosted foods in the same cabinet, but objections might be raised if bulk ice cream were dispensed from the same cabinet. W.C.C.

16. Testing Mix Ingredients. D. LEVOWITZ, Ice Cream Field *32*: 8, pp. 39–40, August, 1938.

The author briefly outlines some of the more obvious characteristics of part of the common ingredients used in ice cream indicating certain precautions such as cooling, etc., which should be followed in preserving their quality. Limitations of bacterial contents of acceptable quality are given for the products discussed. W.C.C.

17. Package Ice Cream. KEN FORREST, Ice Cream Field *32*: 7, 6, 8, July, 1938; Ice Cream Field *32*: 8, pp. 9–11, August, 1938.

Proper selection of color and design are emphasized in choice of packages for ice cream. The author also warns the manufacturer against letting dealers dispense his ice cream in unidentified packages. He claims further that every effort should be made by the manufacturer to build up name preference with the consumer.

The author cites many examples in which ice cream has been successfully merchandized in factory filled packages. W.C.C.

MILK

18. Electric Pasteurization Feature of Air Conditioned Pine Grove Dairy. ANONYMOUS. Milk Dealer, *28*: 1, pp. 68–70, October, 1938.

A description of the Pine Grove Dairy, Schenectady, New York, in which has been installed a stainless steel milk pasteurizer of a new design.

No external heat is applied to the milk. Electricity generates heat right in the milk itself. In 45 seconds' time, milk previously cooled to a temperature of 50° F. or less is raised to a pasteurizing temperature of 162° F., held at that temperature for 15 seconds, and then cooled again to 50° F. or less and delivered by gravity to the bottling machine. Operation is automatic and continuous. C.J.B.

19. The Manufacture of Pump Churned Cultured Buttermilk. George Sauer, Los Angeles, Calif., and L. V. Towt, Oakland, Calif. Milk Dealer, *28*: 1, pp. 76–82, October, 1938.

Detailed procedure is given for the manufacture of churned buttermilk in which a specially designed centrifugal pump is used in place of a better churn.

It is concluded that the quality of buttermilk is determined by the quality of the starter and that the churned flavor and aroma is the by-product of the citrovorus and paracitrovorus organisms in the starter and not the absorption of a churned flavor from the churn. C.J.B.

20. "Use of Milk" Survey Completed in City of Chicago. Anonymous. Milk Dealer, *27*, 12, pp. 62–68, September, 1938.

The research bureau of De Pauw University, after personally interviewing 7,170 families in the City of Chicago, in a "use of milk" survey, summarize their findings as follows:

Quite a large percntage of families interviewed had boarders or outsiders living with them—about 19.4 per cent, and the number having children under 16 years was rather small. Average number of quarts of milk consumed by families of two, three, and four is much lower than government estimates for the actual needs of such families. The average for Chicago families of two was 5.82 quarts, for families of three it was 9.28 quarts, and for a family of four was 10.93 quarts. The government estimate for requirements of such families was: family of two, 7 quarts or its equivalent in other milk products, family of three, 14 quarts or its equivalent, and a family of four, 17 quarts, or its equivalent. The average for all families 7,170 was 11.63 quarts weekly, and the average sized family was 4.7 persons.

Milk prices in Chicago showed wider variation than average prices for the whole United States. The Chicago price had a tendency to go up faster than the average. This was, also, true of other large cities. Notwithstanding this relationship of the price of milk in large cities, the conditions affecting the cost of milk vary much for different cities such as New York and Chicago. Furthermore, the housewife has not been sold on the price of fluid milk delivered, bought at grocery stores, or bought at cash milk stores.

Some rather interesting information was brought out regarding seasonal fluctuation in sale of milk. It was found that the peak month (October) in Chicago is not the same for New York or Boston, and other very large cities. The nationality of the people living in certain cities would have some influence on milk consumption. Cities with a large preponderance of Irish and Germans would show a greater consumption per capita. The Irish rank first, and the Germans second as milk drinkers. The native whites come third. The Negro drinks less milk than any other group in this country.

A smaller number of housewives were buying cream than in previous surveys. It was found that approximately 25 per cent bought cream from cash milk stores. Native whites used the most cream per day; the Irish were a poor second, and the Germans were third.

The housewife is not sold on 13 cents a quart for milk. No mention was made that milk is any better than in other nearby cities where the price of milk is lower. 2,804 out of 7,170 housewives said they would buy more milk if it was cheaper. They probably had in mind more than one cent decrease in price.

Reasons why the housewife preferred one company over another were: first, quality; second, service; third, flavor; fourth, buttermilk; etc.

C.J.B.

21. **Composite Sampling of Milk and Cream.** S. CRAIG ALEXANDER. Milk Dealer, *27*, 12, pp. 38, 48, September, 1938.

A proportional sampling device tested and approved by the Department of Agriculture of the State of California is provided with four scales on a dial. These are so arranged with respect to the retaining or sample chamber (with which they are directly connected through the connecting rod) that when a patron's selected scale (on the dial) is set at the weight of the shipment as shown on the weighing scale, the individual sample taken is always proportionate to the weight of the shipment sampled, and the accumulated total will be within the range of volume of the composite sample desired. Setting the scale is extremely simple. C.J.B.

22. **What's Wrong with Fluid Milk Markets?** CHESTER FLETCHER, R. R. 3, Waukesha, Wis. Milk Dealer, *27*, 12, pp. 36–37, 73, September, 1938.

A review of the trend in delivery and plant operating costs. The author concludes that in the fluid milk business the sales will continue to drift downward and the producers' share of the consumers' dollar continue to decline until labor is compelled to meet the producer on equal terms—receive pay for what they actually do and nothing else. C.J.B.

23. **Insulated Milk Tanks.** ANONYMOUS. Milk Dealer, *27*, 12, pp. 34–35, 68, September, 1938.

A complete description of the insulated milk-cooling tank which is being constructed throughout the Milwaukee milk shed. C.J.B.

24. Ultra-Violet Ray Pasteurization of Milk. S. Mancovitz, Reiter Dairy Co., Akron, Ohio. Milk Dealer, *27*, 12, pp. 33, 58, September, 1938.

It was found possible to sterilize milk in less than 45 seconds' exposure to ultra-violet ray. Such milk, however, was unmarketable due to off-flavors. Using an average time of 19 seconds, an average reduction in bacterial counts from 69,000 per cc in the untreated raw milk to 4,500 per cc in the treated milk was obtained. The taste and smell of the milk was not affected by the shorter time exposure. While these results were obtained with practical large volumes of milk, further extensive work is required to indicate the practical potentialities of ultra-violet ray pasteurization. C.J.B.

Editor's note: It is questionable whether this process should be called pasteurization.

25. The Consumption of Dairy Products in Burlington, Vermont (1935–36). H. B. Ellenberger and J. T. Stearns, Vermont Agric. Exper. Station, Burlington, Vt. Bull. *433*, May, 1938.

The consumption of fluid milk and other dairy products, per family and per capita, in Burlington, Vt., during 1935–36, was determined by a house-to-house survey. All sections of the city were covered by this survey which included 3,616 families and 15,258 persons or about three-fifths of the population. The following summary sets forth the percentage of all families using the different products and the average consumption per family and per capita for all families and for the families that used the product.

Per capita and per family consumption of dairy products

	Percentage of families using the product	Average consumption			
		Families using		All families	
		Per family	Per capita	Per family	Per capita
Fresh milk, pints daily	94.8	4.10	.97	3.89	.92
Canned milk, tall cans weekly	35.0	3.81	.84	1.27	.30
Heavy cream, ½ pints weekly	56.7	2.54	.62	1.44	.34
Butter, pounds weekly	90.7	2.66	.64	2.41	.57
Butter substitute, pounds weekly	14.0	2.57	.48	.36	.09
Cheese,* pounds weekly	61.9	.90	.21	.56	.13
Milk equivalent, pounds yearly	100.0	5,017	1,189	5,017	1,189

* Including cottage cheese.

In addition it shows the variations in rates of consumption by different families according to income, size, nationality, etc., and discloses the more important reasons for restricted usage as well as consumer reactions to quality, price, etc. J.M.F.

26. Factors Causing Variations in Milk Returns. C. G. McBride, Ohio Agr. Exp. Sta., Wooster, O. Ohio Exp. Sta. Bimonthly Bull. Vol. XXIII, No. 193, July–August, 1938.

The following factors causing variation in milk returns are discussed: prevailing market prices, butterfat content, seasonal production, marketing plans, special market, transportation costs, and financial responsibility of buyers. W.E.K.

MISCELLANEOUS

27. Progress of Agricultural Research in Ohio, 1936–1937. Ohio Agricultural Experiment Station Bull. 592, 142 pp., 1938. (Forty-sixth annual report.)

Brief summaries are presented of the results obtained in many projects, of which the following are of particular interest.

Livestock versus grain farming. C. G. Williams (pp. 24–26).

Palatability of soybean oil meals. C. F. Monroe (p. 77).

Peas, oats, and flax for cows. C. F. Monroe and L. E. Thatcher (pp. 77–78).

A method for measuring the loss of juice and volume-weight relationship at different pressures of ensiled crops. A. E. Perkins (pp. 78–80).

Corn-soybean silage versus alfalfa hay and corn silage. C. C. Hayden, C. F. Monroe, W. E. Krauss and A. E. Perkins (p. 80).

Loss of carotene in haymaking. W. E. Krauss and R. G. Washburn (p. 81).

A study of the hydrogen-ion concentration of the contents of the bovine rumen. C. F. Monroe and A. E. Perkins (pp. 81–82).

The determination of ammonia and urea in milk. A. E. Perkins (p. 82).

Whole milk and magnesium carbonate for bone growth in dairy calves. C. E. Knoop, W. E. Krauss, and C. C. Hayden (p. 83).

Raw versus pasteurized milk. W. E. Krauss and R. G. Washburn (pp. 83–84).

Pasture milk versus dry feed milk. W. E. Krauss (pp. 84–85).

Studies on the vitamin C content of milk. R. G. Washburn and W. E. Krauss (pp. 85–86).

Effect of vitamin A on mineral assimilation. W. E. Krauss, C. F. Monroe, and R. G. Washburn (p. 86).

Studies on the absorption of ultraviolet light by dairy calves. W. E. Krauss and C. E. Knoop (pp. 87–88).

Effect of ultraviolet light on the health of dairy cattle. W. E. Krauss, B. H. Edgington, C. F. Monroe, and C. C. Hayden (pp. 88–89).

The artificial induction of oestrus in the milk goat during the anoestrus period. T. S. Sutton and W. R. Krill (p. 89).

The effects of vitamin A deficiency on reproduction. T. S. Sutton and J. B. Brief (pp. 90–91).

The stability of carotene and vitamin A in a mixed ration. R. M. Bethke, P. R. Record, and O. H. M. Wilder (p. 99).

The loss of carotene in dehydrated alfalfa leaf meal stored under different conditions. O. H. M. Wilder and R. M. Bethke (p. 100).

Mastitis in cattle. A. J. Schalk, B. H. Edgington, and T. S. Sutton (p. 103).

Effect of mechanical processing of feeds on mastication and rumination. C. H. Kick, Paul Gerlaugh, A. J. Schalk, and E. A. Silver (pp. 103–105).

Nutritional status of college women in relation to their dietary habits. H. McKay and M. B. Patton (pp. 108–110).

Effect of milk marketing plans on return to producers. C. G. McBride (p. 112).

Changes in volume of milk deliveries by individual milk producers. R. W. Sherman (p. 113).

Factors influencing farmers to market cooperatively. G. F. Henning (pp. 115–116). W.E.K.

28. The Use of Metals in the Dairy Industry. II. Nickel and Its Alloys.

G. Genin. Engineer E. P. C. I. Paris, France, Le Lait *18*: No. 178, 785–805, September–October (1938).

A review is given in which the following phases of the subject are discussed; physical, mechanical and chemical properties of nickel, Monel metal and Inconel, commercial forms of nickel and its alloys, corrosion of nickel and its alloys by milk and by washing and sterilizing solutions and the influence of nickel on the food value of milk including the vitamins. A.H.J.

29. The Function of Collecting Womans' Milk at Erfurt.

Marie-Elise Kayser. Le Lait *18*: No. 178, 805–812, September–October (1938).

In some 700 mothers from whom "surplus" milk was collected, the quantities available varied from 150 grams to 1500 grams per day. The quality of the milk was controlled by determining the acidity. The normal acidity of mothers' milk is much lower than that of cows' milk. Thus mothers' milk has an acidity of 1 to 2 degrees S–H, pH 7.25–6.94, while that of cows' milk is 6 to 8 degrees S–H. The fat content of the mothers' milk that was collected varied between 0.6% and 9.7%. Mothers' milk generally has a fat content of about 3.2%. Methods of distinguishing between mothers' milk and cows' milk are discussed. When exposed to solar light in a special glass container mothers' milk gives a blue fluorescence while under the same conditions cows' milk gives a yellow fluorescence. By using the biologic serum method developed by Koschucharoff the presence of 5% of cows' milk can be readily detected in mothers' milk. Mothers' milk above the supply required is made into dry milk powder. A.H.J.

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New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

30. Cattle Fodder and Human Nutrition. Arturi I. Virtanen. Cambridge University Press; New York, The MacMillan Co., 108 pages, illus. 1938. $2.25.

This book comprises four lectures the author delivered at the Universities of London, Reading, and Cambridge during 1937. The lectures were: I. The Mechanism of the Biological N-Fixation, II. The Symbiosis of the Leguminous Plants with the Legume Bacteria, III. The Production of Vitamins in Agriculture, with Special Reference to Human Nutrition, and IV. The A. I. V. Method for the Preservation of Fresh Fodder and Its Importance in Agriculture. The appended General Survey summarizes and correlates the material of the four lectures which might otherwise have appeared somewhat unrelated. Included in the Survey is a diagram of the author's conception of the cycle of nitrogen changes as related specifically to the fixation and excretion of nitrogen by plants and the utilization and excretion of nitrogen by animals.

The author, Professor at the Biochemical Institute at Helsinki, Finland, is eminently qualified to discuss the material presented in this small volume. He has initiated and directed many investigations which have originated or stimulated the development of new techniques for the study of nitrogen fixation problems.' Of equal importance have been his studies resulting in the development and practical application of new methods. His statements and conclusions regarding the A. I. V. method of ensiling are scientifically correct and have repeatedly been verified by other scientists working under like conditions. Climatic conditions in this country permit the ensiling of the various crops at a higher dry matter content thereby preventing any loss of juice from the silo when proper precautions have been taken. For this reason the acid treatment of silage crops is of less significance in this country than in western Europe. However, in our experience the A. I. V. process is probably entitled to the highest rank among the various preservative treatments for forage crops with respect to the preservation of the carotene content of the green crops.

In his own country he has been unusually successfull in making extensive practical use of his investigations and he has inspired much recent work in other countries.

Although the author includes a general review of the recent literature in the fields discussed, undoubtedly his readers will welcome particularly his evident attempt to make available in the English language the methods and conclusions of his own investigations, thus far too little known. With slight modifications, the General Survey might well have formed an Intro-

duction to the book. The material has been carefully organized and well illustrated. A more appropriately chosen title would undoubtedly have resulted in the book receiving a more wide-spread and well-deserved reception.
A.E.P. and H.W.B.

31. **Casein and Its Uses.** HANS HADERT. (Translation of second German edition by Dr. Henry Goldsmith.) Chemical Publishing Company of New York, 1938. Price $3.00.

In this book of slightly more than 100 pages the author has assembled a surprisingly large amount of useful and practical material on the manufacture and uses of casein. The book is written from the viewpoint of a technical expert in this field, and as its translator states in his preface it should be a valuable handbook for technicians in the casein industry and to those interested in the industrial uses of this product.

The first chapter covers briefly the various processes of manufacture of both acid precipitated and rennet type caseins. Flow sheets are given and typical plant operations are described. Factors affecting the quality of the product are discussed. Since the author is acquainted chiefly with German plants most of the machinery mentioned is obviously of German make, but the operations described are, of course, similar to those used with American machinery. German standards for casein and methods of testing casein are given in detail.

Some thirty pages are included in Chapter II—"Casein Adhesives." The grades or casein to be used, factors affecting viscosity, action of the numerous possible dissolving agents, waterproofing ingredients, etc., are discussed. This chapter assembles a large number of facts of practical value from a wide survey of the field, and gives numerous references to the literature as recent as 1936. Some attention is given to adhesives made by mixing other animal and vegetable proteins with casein, such as blood albumin, soy bean protein, ivory nut flour, castor bean protein, etc. The patent literature of this field in Germany and other countries seems to have been extensively covered by the author in his survey. Numerous practical recipes and mixes are given in detail for the preparation of adhesives for specific purposes.

Casein paints and color binders are the subjects of the third chapter. It is given over to a discussion of the various ingredients which may be used in the manufacture of these products. Again a large number of recipes is given, and a wide survey of the latest improvements and newest ingredients suggested in this field is condensed into a brief space, thus making available to the reader the most recent developments.

The concluding chapter—"Other Applications of Casein"—touches briefly on the use of casein in paper making, in plastics, textiles, medicines, food products, etc., mentioning also recent work on the textile fiber, "Lanital." Most of this chapter, however, is given over to some 30–40 miscellaneous formulas using casein in various mixtures to be used for various purposes from paper sizing to cellular concrete mix.

As a whole the book accomplishes what the author intended in furnishing a handy and compact technical reference summarizing the industrial phases of the manufacture and use of casein. However, most of the emphasis is placed upon adhesives and paints as the major casein using industries, and persons interested in casein plastics, or other casein products will find less use for the book in their particular fields.

This edition of the book may be criticized somewhat on two points. The translator seems to have clung a little too closely to the literal translation at many points, which results in some rather unusual English equivalents; indeed, in some cases, in actual misleading statements. The second point is that the whole book gives one the impression of having been printed without the benefit of careful proof-reading. These are minor matters, however, and the book should prove of general interest to all who are interested in the casein industry, and particularly to manufacturers and technicians in the adhesive and paint phases of it. E.F.A.

32. **Utilization of Fats.** A Theoretical and Technical Treatise on the Composition, Analysis and Applications of Fats. H. K. Dean, with a foreword by T. P. Hilditch. 292 pages, Chemical Publishing Company, 148–H Lafayette Street, New York City. 1938. Price $6.00.

The industrial applications of fatty materials and their derivatives are described on the basis of a physico-chemical approach. The primary consideration is to present a clearer picture of the relation of structure of fats to their utilization, achieved largely by the replacement of the usual tables of iodine, saponification and other average values and the classification based upon them by tables showing the composition of fats in terms of fatty acids, and where possible glycerides. Titles of the seven chapters are: Constituents and Properties of Fats; Analytical Methods; Classification, Composition and Analysis of Fats; Artificial Fats; Extraction and Refining of Fats; Edible Fats; Paint and Varnish Oils; Soaps and Fatty Acids; Miscellaneous Applications, including Pharmaceutical Fats, Cosmetics, Lubricants, Leather and Sulfonated Oils. The inclusion of the fat soluble vitamins and the rancidity problem extend the interest of the book to a wider circle. J.F.L.

FOOD VALUE OF DAIRY PRODUCTS

33. **Is Cobalt of Any Significance in the Treatment of Milk Anemia with Iron and Copper?** E. J. Underwood and C. A. Elvehjem, De-

partment of Biochemistry, University of Wisconsin, Madison. J. Biol. Chem. *124,* 419, 1938.

Cobalt in exceedingly minute quantities is active in animal metabolism, and in comparatively large doses induces polycythemia.

It was concluded from the study that iron, copper and manganese are the only minerals which must be added to milk under the conditions of the experiment for the normal growth and well being of the rat, and that small amounts of cobalt, which contaminate almost all iron salts, play no significant part in the treatment of milk anemia in the animal. K.G.W.

34. Soybean Products as Competitors of Dairy Products. SYBIL WOODRUFF, Univ. of Ill. Nat. Butter and Cheese Jr. Vol. *29,* No. 20, p. 30, Oct. 25, 1938.

Soybean contains 40% protein and 20% oil, approximately. Crushing 100 pounds of soybeans yields about 12.5 pounds of oil. The uses of this oil as a food over a period of five years is indicated in this table.

Factory Consumption of Soybean Oil for Food and Industrial Uses Over a Period of Five Years

Year	Lard compounds and vegetable shortenings	Margarines	Other edible products	Total	Total industrial uses
	Thousand pounds	Thousand pounds	Thousand pounds	Thousand pounds	Thousand pounds
1932	4,889	3	180	5,072	19,039
1933	489	7	460	956	21,135
1934	2,735	24	509	3,268	16,816
1935	52,452	1,740	9,421	63,613	22,085
1936	113,897	14,262	21,598	149,757	25,847

Refined oil may show flavor changes, sometimes called fishy, that restrict its saleability as food. Six pounds of soybean press cake are produced with every pound of oil. It is used chiefly for livestock, but some is ground into flour or coarse grits for human consumption. Several varieties of soybeans are used as vegetable either green or dried. Soybean milk resembles cow's milk in appearance and contains proteins, carbohydrates and minerals which are extracted from ground, raw soybeans with water. Although the flavor is strange the milk may be used for cooking purposes and has been used by people allergic to cow's milk. Although the food uses of soybeans may be extensive according to present knowledge of its nutritive value soybean protein can be advocated only as a supplement to animal proteins.

W.V.P.

35. Foods, Fads, and Fallacies. Tate Miller, M.D., Dallas, Texas. Ann. Conv. Intern. Assn. Milk Dealers, Sales and Advertising Section, p. 73, 1937.

With the exception of the dietary treatment of diabetes and the diet of babies 90% of what the general public thinks about diet is pure bunk. The advertising claims for 400 different foods causes the consumer to wonder what he should eat. One cannot be guided by advertisements as to food or health directions. This is an interesting and readable discussion of food, fads, and fallacies. E.F.G.

ICE CREAM

36. Vanilla "As They Like It." T. B. Harrison, Univ. of Tennessee. Ice Cream Trade J., pp. 34, 10, 25, October, 1938.

Consumer preference tests were made at the University of Tennessee on ice cream submitted at different times by Tennessee manufacturers. Fat, total solids, acidity tests were made and flavor, body and texture defects noted. The testers were requested to place the samples in order of their preference. Five such tests were made with 57 to 73 people judging each set of samples.

Flavor proved to be the most important factor in the consumer choice of sample. Outstanding flavor defects were usually identified by the consumer and resulted in a low rating. In some instances pronounced pure vanilla flavor seemed to be preferred to a flat or unnatural vanilla flavor. Samples with too deep color and high acidities were placed down. The fat content of the samples ranged from 9 to 18 per cent, and had some effect on consumer preference but not as marked as flavor and body and texture defects. The type preferred by the majority of consumer judges was described as that having a true vanilla flavor, free from off flavors of dairy products, smooth in texture, low in acidity, and a pale yellow color.

W.H.M.

37. Malted Milks. Mal Parks. Ice Cream Trade J., pp. 34, 11, 20, Nov., 1938.

Salesmen who suggest to their dealers that they lower the price of malted milks will usually find that the dealer cuts down on the amount of ice cream used in each serving and reduces the quantity and quality of other ingredients used.

The making of a good malted milk requires that all ingredients must be fresh and pure, the proper amount of each ingredient should be used, the milk must be very cold, and the drink must be allowed to mix for a suitable length of time.

Directions given for a good malted milk are as follows: 1. Put 1.5 oz. of syrup into a clean, shining mixing cup. 2. Add 6 oz. of very cold milk. 3. Add 2 No. 20 scoops of ice cream and ½ oz. malted powder. 4. Mix at least 1 minute. 5. Serve in a clean sparkling glass, dressed with whipped cream, placed on saucer, with a doily and malted milk cookies. The cost price on this malted milk is estimated to be 10¼ cents. W.H.M.

38. Specialties. J. H. FRANDSEN, Mass. State College, Amherst. Ice Cream Trade J., pp. 34, 11, 29, Nov., 1938.

The author suggests many ways of increasing ice cream sales. A list of special forms of ice cream which can be used for many occasions throughout the year is presented. W.H.M.

39. The Importance of Quality Syrups. MALCOLM PARKS. Ice Cream Trade J., pp. 34, 10, 70, October, 1938.

It has been found practical for the ice cream manufacturer to supply the dealer with paper and glass containers for sundae topping to be sold with ice cream for home use. This plan has stimulated the bulk package business in many stores. W.H.M.

MILK

40. A Survey of the Progress of Pasteurization. JAMES A. TOBEY, The Borden Company, New York City. Ann. Conv. Intern. Assn. Milk Dealers, Sales and Advertising Section, p. 90, 1937.

The history of pasteurization of milk is reviewed and the present extent of the practices is evidenced by the fact that in 1931 there were 99 cities in which all milk except certified was pasteurized. It is stated that pasteurization offers a guarantee of the safety of the milk supply which will increase consumption. E.F.G.

41. The Sales Possibilities of Homogenized Milk. W. A. MACDONALD, Pres., MacDonald Dairy Co., Flint, Mich., Ann. Conv. Intern. Assn. Milk Dealers, Sales and Advertising Section, p. 99, 1937.

After analyzing the milk sales problem opportunities for improvement will be uncovered. The advantages in looks, taste, fat distribution, and soft curd character give the route man a new approach to the customer. It is recommended that homogenized milk be sold as soft curd milk. Educational advertising to groups proved especially effective. Starting with 6% of sales in 1933 distribution increased to 25% of sales in 1937 with homogenized milk selling at a 1¢ premium. E.F.G.

42. Composition of the Milk from the Breasts of Newly Born Infants. W. L. Davies and Allan Moncrieff. National Institute for Research in Dairying, University of Reading, and Middlesex Hospital and Queen Charlotte's Maternity Hospital, London. Biochem. J. *32,* 1238, 1938.

Most infants of both sexes secrete milk when newly born, the secretion being known as witches' or sorcerers' milk. The analyses of five samples of secretions from the breasts of newly-born infants from 8 to 14 days old showed the following values: total solids 7.4–12.5 per cent, consisting mostly of protein and lactose. Compared with normal milk, the secretions are low in lactose but high in globulin and non-protein nitrogen. The secretions contain appreciable amounts of peroxidase and phosphatase. K.G.W.

43. Ascorbic Acid and Phosphatase Activity. E. J. King and G. E. Delery, British Postgraduate School, London. Biochem. J. *32,* 1157, 1938.

It was reported in 1937 (J.B.C. 121, 697, 1937) that ascorbic acid was an intense activator of serum phosphatase. A repetition of these experiments, as well as others, in which significant quantities of ascorbic acid were added to serum, plasma or substrate indicated that ascorbic acid is without effect on the activity of phosphatase. K.G.W.

44. New Ideas in Milk Drinks. J. H. Frandsen, Mass. State College, Amherst, Mass. Extension Leaflet No. *177,* July, 1938.

Directions are given for making many milk drinks by the addition of fruit juices and other flavoring materials to milk. H.G.L.

45. The Sales Possibilities of Homogenized Milk. James R. Hudson, Baker-Hubbell Dairy, Inc., Peoria, Ill. Ann. Conv. Intern. Assn. Milk Dealers, Sales and Advertising Section, p. 111, 1937.

The advantages of homogenized milk are listed, and a program of public, medical profession, and salesman, education is outlined. A radio and sales promotion contest is described. It is held that the amount of homogenized milk sold will be in proportion to the effort put forth. E.F.G.

MISCELLANEOUS

46. How to Control Insects in the Milk Powder Plant. C. L. Fluke, Univ. of Wis. Nat. Butter and Cheese Jr. Vol. *29,* No. 15, p. 30, Aug. 10, 1938.

Accumulations of powdered milk in obscure corners or on inaccessible ledges in milk powder plants encourage insect infestation. The most important insects which use such milk for food are known as carpet beetles (dermestids) such as the black carpet beetle and the Trogoderma beetle. Silver fish (fish moth) may also be found in milk powder factories wherever milk powder is allowed to accumulate. The life histories of these insects are briefly outlined. The prevention and control of these insect infestations is accomplished through general sanitation and cleanliness in the factories. Common places of infection and the most dangerous times of the year are indicated. W.V.P.

47. Cold Storage Locker Plants. T. Edwin Thomas. Nat. Butter and Cheese Jr. Article I, Vol. *29*, No. 13, p. 10, July 10, 1938. Article II, Vol. *29*, No. 14, p. 6, July 25, 1938.

Article I. The five year development of the cold storage locker idea from an unheard of industry to one with two thousand cold storage locker businesses and investment of nearly $25,000,000 is described. A diagram of a typical cold storage locker plant lay out is shown.

Article II. A cold storage locker plant is described and photographs are presented. Operating expenses for a three hundred and twenty locker plant are itemized. The author estimates a return of 10% on the investment. W.V.P.

48. The Value of Contests in Stimulating Continued Sales Efforts. Earle W. Beebe, General Sales Manager, Arden Farms, Inc., Los Angeles, Calif. Ann. Conv. Int. Assn. Milk Dealers, Sales and Advertising Section, p. 58, 1937.

Most effective incentives are discussed. Prize money is held to be no incentive but other prizes are effective in causing a man to want to sell. A milk company can afford to spend all the increased gross profit during the effort on prizes. Results of four summer campaigns and the methods of calculating points are given. E.F.G.

49. Some New Approaches to Accounting for Distribution Costs. Philip Saunders, Pasteur, Charles F. Rittenhouse and Co., Boston, Mass. Annual Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 69, 1937.

The fact that in the milk industry selling and delivery alone accounts for about half of all expense requires that these costs be analyzed and controlled. Many uncontrolled items need to be taken into account in this analysis. Typical milk plant situations and unit costs are given.

E.F.G.

50. Are Vehicle Records Worth the Cost of Compilation? F. W. ROOT, Sales Manager, Glendale Farms, Inc., Wilkes-Barre, Pa. Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 63, 1937.

The records are discussed and a classification of items is given.
E.F.G.

51. A Case Study in Accounting for Capital Assets and Depreciation. JOHN H. DEVITT, Asst. Auditor, Hammermill Paper Co., Erie, Pa. Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 41, 1937.

A punch card system with code numbers has been used effectively in classification and control. This system is described and attention called to its advantages. E.F.G.

52. A Program of Control of Office Plant Forms. CHARLES A. ARMITAGE, Comptroller, United Farmers Cooperative Creamery Association, Boston, Mass. Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 32, 1937.

Eliminating needless reports and simplification of others will cut expense without loss of essential information. How forms may be made more usable is discussed. E.F.G.

53. The Present Status of Social Security Taxes from the Standpoint of Employer Administration. C. J. CRAMPTON, Executive Secretary, Dallas Chamber of Commerce, Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 8, 1937.

The general application of this legislation to business is considered including accounting methods and responsibilities of the employer.
E.F.G.

54. Interpreting General Statistics for Dairy Industry Executives. H. A. ROSS, Head of Bureau of Economics, The Borden Co., New York City. Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 106, 1937.

Sources and analysis of statistics to answer the following questions are presented

1. What will milk production be?
2. What will be the price for milk?
3. How will general business affect sales?

A comprehensive list of sources of statistical information is given. Suggestions for interpretation are offered. E.F.G.

PHYSIOLOGY

55. Studies in the Secretion of Milk Fat. I. The Effect of Inanition on the Blood Lipoids of the Lactating Cow. J. A. B. SMITH. II. The Effect of Inanition on the Yield and Composition of Milk Fat. J. A. B. SMITH AND N. N. DASTUR, Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland. Biochem. J. *32,* 1856, 1938.

During the past few years evidence, obtained by simultaneous arterial and venous blood sampling, has shown that the only lipoid fraction of the blood used by the mammary gland for milk fat production is the neutral glyceride fraction and not that comprising the phosphatides or cholesteryl esters. This might suggest that for the secretion of milk fat the gland simply selects from the blood the particular type of glycerides which it requires and that these are then allowed to diffuse into the milk. But it is clear from the composition of the fat itself and from measurements of the activity of the gland that more complex processes, such as synthesis of fat from carbohydrates, are involved. It would appear there are at least two main precursors of milk fat in the blood, the neutral glycerides on the one hand and some form of carbohydrate material on the other. It seems reasonable to suppose the composition of milk fat produced by an individual animal at any particular time will depend on the extent to which each of these precursors contributes toward its production. These two papers are in part a report of experiments to determine the effect of altering blood composition of milk fat.

Inanition of three cows resulted in very slight decreases of considerably less than 10% for the lipoid levels in the corpuscles, during and after a 12-day fast, whereas for the plasma, general reductions of as much as 40% or 50% took place and these low levels persisted even six weeks after free access to food had again been permitted. Neither in the corpuscles nor in the plasma could any appreciable change be detected in the actual nature of the lipoids as a result of inanition, although there was a tendency for greater unsaturation in the plasma fatty acids to accompany milk fever symptoms. It was also shown that if any water soluble fatty acids are present in the non phosphatide fraction of the plasma the amount must be exceedingly small.

Although no appreciable change in the nature of the blood lipoids of the lactating cows during inanition was observed, very considerable changes were observed in the milk fat. The chief change was a decrease of about 80% in the original content of lower acids up to and including C_{14}, a deficiency which was almost entirely made good by an increase in the content of oleic acid. A concise review of the theories relating to fat secretion in the light of the above data is included.

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ABSTRACTS OF LITERATURE

BOOK NOTICE

Elements of Dairying. T. M. Olson. 570 pp., illustrated, price $3.50. The Macmillan Co., New York.

Elements of Dairying is a survey of the Dairy Industry intended for use as a textbook in the first course in dairying. It is divided into six parts under the headings, "The Dairy Industry," "Dairy Cattle," "Milk," "The Processing Industries," "Feeding Dairy Cattle," and "Dairy Herd Management."

This book "intended to be used as an introduction and not as an exhaustive treatment" should experience enthusiastic acceptance. It covers a field in which the number of available texts have been quite limited in recent years. In addition to serving as a text for the first course it should be equally valuable for groups of students who take only one course in dairying, for example students majoring in Agricultural Education or Agricultural Extension.

This work is well illustrated and well written. Many of the more recent developments in the dairy industry are presented. A unique feature in the appendix is a history and informative discussion of each of the national dairy organizations.

G.W.B.

BACTERIOLOGY

56. The Respiration of the Rod-Shaped Lactic Acid Bacteria. P. A. Hansen. Zentralbl. f. Bakt. *98:* 289–297, 1938.

Twelve representative strains of lactobacilli (Thermobacteria, Streptobacteria and Betabacteria) and microbacteria served for an inquiry of the respiratory mechanism. All experiments were carried out in a Srensen Phosphate buffer solution with the Warburg respiration apparatus. Results in brief:

Qo_2 of glucose varies from 1 (*Thermobacterium cereale* and *helveticum*) to 124 (*Microbacterium flavum*). *Thermobacterium* and *Streptobacterium* have no hemin in contradistinction to *Microbacterium.*

It is recommended to consult quantitative respiration experiments for taxonomic purposes. K.J.D.

57. Contributions to the Knowledge of the Reduction of Cow's Milk with Reference to the Reduction Mechanism. H. O. Jensen. Zentralbl. f. Bakt. II, *96:* 234–257, 1937.

Reduction of methylene blue in *pure cultures* of lactic acid streptococci, micrococci and sporeformers is strongest, but more cells are needed in raw milk to get the same effect. Alkali formers show a very slow reduction. The reduction power ceases in general as the cells grow older (retarding effect of lactic acid).

Reduction of methylene blue in *raw milk:* The most active reducing germs are the lactic acid streptococci; *B. coli* and micrococci, however, exert practically no influence. The bactericidal power does not materially retard the decoloration of the dye.

The reduction is intensively connected with the energy metabolism of the cell and the author discusses the relation of it to the theory of dehydrogenation of Wieland.

The reductase test is only useful for certain limited purposes, besides that it must be combined with other tests. K.J.D.

58. Dye Concentration in Culture Media Employed for the Analysis of Escherichia-Aerobacter Members in Milk. HERBERT D. MCAULIFFE and M. A. FARRELL, Div. of Bacteriology, Pennsylvania State College, State College, Pa. Am. Jour. Pub. Health. *28:* 10, 1217, 1938.

The concentration of the bacteriostatic agents in fuchsin lactose broth and in brilliant green lactose bile broth 2.0 per cent was developed for use in water analysis. Addition of 1 ml. portions of milk to these media adsorbed a considerable amount of the dyes present, thereby lessening the selective action of these media in the detection of the *Escherichia-Aerobacter* group of organisms in milk.

The authors determined the concentration of the free dye present after adsorption and found that the content of basic fuchsin in fuchsin lactose broth should be increased from 1 part in 66,666 to 1 part in 9,500, and the content of brilliant green dye in brilliant green lactose bile broth 2.0 per cent increased from 1 part in 75,188 to 1 part in 30,000. With these modifications, false presumptive tests were largely eliminated without lessening the selective properties of these media for the *Escherichia-Aerobacter* group.
M.W.Y.

BREEDING

59. Midget Cows. GERALD BENEDICT. Nat. Butter and Cheese Jour. *29:* 21, 27, 1938.

Otto Gray, Stillwater, Oklahoma, has developed a strain of midget cows ranging in height from thirty inches to thirty-seven inches. Leg length is twelve inches. The midget weighs four hundred to five hundred pounds and produces from thirty-five to forty-one pounds of milk per day. Forty-one pounds of this milk produced two and one-thirds pounds of butter.
W.V.P.

CHEESE

60. Bacteriological and Chemical Examinations of Normally and Badly Ripened Tilsit Cheese. W. Meyer. Zentralbl. f. Bakt. II, *98:* 212–232, 1938.

The *normally* ripened Tilsit cheeses of different make did not show very many discrepancies regarding the microflora: After a primary acceleration of the growth of the streptococci the streptobacteria were developing and did not decrease until after the second month. The streptobacteria have a significant proteolytic power, but this is variable with the different strains. Their microscopic appearance in cheese is streptococcus-like. The thermobacteria do not develop earlier than 5–6 weeks after fabrication and decrease again after the 8th week. The acidoproteolytic cocci were always abundant and play an important rôle in Tilsit cheese ripening. The water soluble and amino-N proved to increase slowly, but constantly during ripening. Cheeses, made from highly buffered milk (addition of resp. salts), showed a materially different microflora as compared with normal cheeses. The lactic acid bacteria and acidoproteolytic cocci were after 2–3 weeks substituted by a flora, which mainly consisted of putrefactive germs and sporeformers. Many other bacteria could be found, which never were detected in normal cheeses. The reaction of the curd changed to the alkali range. The aroma was sweetish-putrefactive and partially bitter. The water soluble N did not show any significant difference in comparison with the normal cheese, but the value of amino-N was much higher with the poor cheeses. K.J.D.

61. Propionibacterium rubrum from Dairy Cheese. M. Lubow. Zentralbl. f. Bakt. II, *99:* 107–115, 1938.

The organism was isolated from rusty spots in cheese. The morphological and physiological characters were those of van Niels *Propionibacterium rubrum* (raffinose and mannitol +). The relation of propionic acid to acetic acid was higher than 2:1. The pigment formation proved to be independent from oxygen. The red dye was very stable and it could not be destroyed by the common special agents, either by hydrochloric, sulfuric or nitric acid. K.J.D.

62. Pasteurization of Milk for Cheese Making. (In two parts.) W. V. Price, Univ. of Wis., Madison, Wis. Nat. Butter and Cheese Jour. *29:* 21, 16, 1938; *29:* 22, 20, 1938.

The commercial application of pasteurization of milk for the manufacture of cheese is discussed. A partial review of the literature includes fifty-seven references. It is indicated that pasteurization of milk for cheese is applicable to the most important types manufactured in the United States excepting Swiss. Pasteurization improves the quality of cheese by eliminating defects caused by undesirable organisms in the raw milk. It improves the uniformity and keeping quality of the cheese and may be regarded as a necessity when conditions beyond the control of the cheese maker endanger the quality or healthfulness of the factory milk supply. W.V.P.

63. **Buys Milk by Grade.** PAUL H. MANDT, Milwaukee, Wis. Nat. Butter and Cheese Jour. *29:* 23, 6, 1938.

The Outagamie Producers'-Consumers' Cooperative, Black Creek, Wisconsin, grades milk for cheese making by taste, smell, sediment, methylene blue and curd test, and places second grade milk in a vat by itself. This milk is made into cheese and producers who furnish the "B" milk are paid for it only what the cheese is worth. The differential in price ranges from one to three cents per pound of butterfat. This program has resulted in the production of over ninety-eight per cent of cheese in the State Brand grade. Record sheets are shown for milk grading, operating statements, cost statements, and daily manufacturing records. W.V.P.

64. **Purpose and Function of Starters in Cheese Making.** H. F. LAABS. Nat. Butter and Cheese Jour. *29:* 21, 28, 1938.

This is a practical discussion of the purposes of using starter and the effects of acid development in cheese making. It is stated that starter inhibits the growth of undesirable bacteria, favors curdling of the milk with rennet, aids in expulsion of whey, favors the fusing of curd particles, aids in the development of cheese flavors and aroma and establishes proper conditions for the mellowing of cheese. W.V.P.

65. **Salting American Cheese for Flavor.** J. L. SAMMIS, Univ. of Wis., Madison, Wis. Nat. Butter and Cheese Jour. *29:* 22, 6, 1938.

A large proportion of the cheese made today does not contain enough salt to be perceptible to the taste. Makers commonly add two and one-half pounds of salt to one hundred pounds of curd but much of this salt is lost in the brine running from the gate and from the press. The author recommends that this amount of salt is sufficient providing the curd is milled into small pieces and these pieces well stirred to keep them from matting and providing the milled curd pieces are wet with brine. If a pail is placed under the open gate at the time of salting to catch the brine from the curd the brine can be poured over the curd and stirred into it every five or ten minutes for an hour between salting and hooping. More emphasis should be placed upon the salt content of cheese by cheese score cards. W.V.P.

FEEDS AND FEEDING

66. Notiz über den Vitamin-Gehalt der Topinamburknollen. (Note on the Vitamin Content of Jerusalem Artichoke Tubers.) A. Scheunert, Veterinär-Physiologischen Institut der Universität Leipzig. Biedermann's Zentralblatt, Abteilung B. Tierernährung. *10:* 4 & 5, 449–450, 1938.

Trials with tubers of the Jerusalem artichoke (*Helianthus tuberosus*) showed that they contain only very small quantities of vitamins B_1 and C. Therefore they cannot be considered of importance as vitamin carriers for either humans or animals. J.G.A.

67. Die Eigung der Kakaoschalen zur Verfütterung an Milchkühe. (The Suitability of Cocoa (cacao) Shells as a Feed for Milk Cows.) A. Hamburg Richardsen. Biedermann's Zentralblatt, Abteilung B., Tierernährung *10:* 4 & 5, 443–448, 1938.

Cocoa shells must be regarded as a substitute feed, consisting only of offal, and of foreign origin. It would be unwise to expect too much from this feed. It may be given to older cattle without risk, but it is not to be regarded as a good dairy feed.

Carob-beans proved to be a neutral food. J.G.A.

68. Ein Beitrag zur Futtervolumenfrage bei wachsenden Rindern, gleichzeitig eine studie über die Aufzucht des Rindes. (A Treatise on the Question of Fodder Volume for Growing Cattle, Coincident with a Study in Cattle Raising.) Hans Brüggemann, Institut für Fütterungstechnik der Versuchs- und Forschungsanstalt für Tierzucht, Kraftborn (Kr. Breslau). Biedermann's Zentralblatt, Abteilung B. Tierernährung. *10:* 4 & 5, 296–384, 1938.

The objective of any feeding standard must be not only the fulfillment of nutrient requirements but the attainment of a state of satiation as well. Satiation is dependent upon the volume of individual feed apportionments.

Although interest in the question of feed volume dates back to the early investigators in animal nutrition, hitherto there has been a divergence of opinion as to methods for ascertaining it. Originally absolute quantity of roughage fed was considered a good measure; later content of dry matter was adopted as a standard, and recently indigestible organic substance ("ballast") has come into use as a criterion of feed volume.

Economic conditions in Germany requiring the extended and increasing use of home-grown roughages, the need for adequate standards respecting feed volume has become more apparent, hence the series of experiments reported here.

Two series of experiments were conducted. The first included several groups of cattle of varying ages during one winter feeding period; in the second series ten heifers were carried through from birth to first calving. All ten were sired by the same bull. In both series feeding was ad libitum, portions unconsumed being weighed back. All feeds were analyzed and the quantities of nutrients consumed were calculated and plotted weekly throughout the duration of the trials.

Results indicated that neither dry matter content nor indigestible organic matter are exact indicators of the volume of any particular ration necessary for satiation, because (1) biological processes of the organism are subject to constant change, and (2) a feeling of satiety is dependent upon somatic stimuli and cannot be judged with absolute objectivity. Differential apportionment of the daily feed allowance gave a better idea of the proper volume of single portions.

Satisfactory growth of the experimental animals, good agreement between individual results and the average, and the fact that the herd at Kraftborn is of well bred black and white lowland stock, warrant the formulation of standards setting forth the proper amounts of feed expressed as single and individual portions for cattle at different stages of growth. These appear in tables and diagrams in the body of the report.

Although the question of feed volume was not solved satisfactorily from a scientific viewpoint, the experiments have demonstrated the soundness of the effort being made in Germany to raise the productivity of cattle by utilization of the resources at hand.

Results of the trials are significantly applicable to practical cattle breeding. Those animals in which there was an especially good development of breadth and depth of the chest, in general showed a high capacity for "ballast" intake and hence for consumption of home grown roughages; further they proved to be especially good feed utilizers.

Attention to these points in selecting animals for breeding would result in an increase in milk yield beyond the present upper limits of production, even if only home grown feeds were fed. J.G.A.

69. Über den Vitamin-A-Gehalt, des Keimöles aus den Samen von Theobroma cacao Linné. (The Vitamin A Content of Germ Oil from the Seed of Theobroma Cacao L.) (The cacao bean.) K. H. Wagner and L. Seber. Veterinar-Physiologischen Institut der Universität Leipzig. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *10:* 2 & 3, 261–264, 1938.

Trials with rats receiving either pure B-carotene or cacao bean germ oil, indicate that 100 grams of the oil contained 825–1400 I.U. of vitamin A.

J.G.A.

70. **Über den Nährwert des A.I.V.-Futters und dessen Beeinflussung durch die Neutralitätsregulation der gefütterten Tiere. (The Nutritive Value of A.I.V. Silage and Its Influence on Neutrality Regulation in the Animals Fed.)** H. MOLLGAARD and G. THORBEK, Tierphysiologischen Laboratorium der landwirtschaftlichen Versuchsstation Kopenhagen. Biedermann's Zentralblatt, Abteilung B. Tierernährung *10:* 2 & 3, 105–121, 1938.

Dry cows were fed with alfalfa dried either naturally or artificially or ensiled by the A.I.V. process. The silage was fed unneutralized, and neutralized with $CaCO_3$, or with $NaHCO_3$. Mineral balances and respiration trials showed that disturbance of the acid-base balance by the unneutralized silage resulted in a marked increase in heat production. The authors conclude from these results that nutritive value of the silage is directly dependent on neutralization, and that the more complete the neutralization, the higher is the net energy value. As complete neutralization of the fodder as possible is therefore recommended.

Neutralization with alkali carbonates always resulted in a lowering of the digestibility of the feed. When neutralization was effected by the feeding of beets this depressing effect was not in evidence, utilization of the nutrients of the fodder being beyond expectation. J.G.A.

71. **Die Bestimmung des allgemeinen Nährwertes (Energiewertes) der Futtermittel nach der chemischen Zusammensetzung. (Estimation of the Energy Value of Feeds from Their Chemical Composition.)** J. AXELSSON, Institut für Haustier-fütterung der Landw. Hochschule Schwedens Uppsala. Biedermann's Zentralblatt, Abteilung B., Tierernährung. *10:* 2 & 3, 238–248, 1938.

The author proposes to determine energy values of feeds as exactly as possible on the basis of chemical composition. Methods are described for adjusting digestion coefficients and feed unit values. Methods are also proposed for the formulation of a sliding scale of deductions according to the relationships between crude fiber, dry matter, and organic matter content of the feed, and taking into account also feed units as well as starch values.
J.G.A.

72. **Milchsäure als Sicherungszusatz bei der Gärfutterbereitung. Ein vergleich zwischen normaler Kaltvergärung und der Vergärung bei Zusatz von Milchsäure, Salzsäure, Schwefelsäure, Zucker. (Lactic Acid as a Safety Factor in Silage Preparation. A Comparison between the Ordinary Ensiling Process and Ensiling with Additions of Lactic, Hydrochloric or Sulfuric Acids, or Sugar.)** H. KLEINERT, Institut für Tierzucht und Milchwirtschaft

an der Universität Leipzig. Biedermann's Zentralblatt. Abteilung B., Tierernährung. *10:* 2 & 3, 122–186, 1938.

Ensiling trials with red clover and alfalfa are described and results noted.

HCl, H_2SO_4, $C_2H_4(OH)COOH$, or sugar added to these protein-rich fodders produced a very good silage. Equivalent quantities of the acids named were about equally efficient for preservation of the fodder. Very fine chopping of the fodder had a very favorable effect.

Conclusions from this and earlier work are:

1. Fodder with ratio between crude protein and N-free extract less than 1:2 (*e.g.*, alfalfa, very young red clover, leguminous mixtures, or young pasture grass) requires 0.4 per cent HCl, or 0.6 per cent H_2SO_4, or 1 per cent of either lactic acid or sugar. A combination of acid and sugar is recommended.

2. Fodder with a crude protein; N-free extract ratio wider than 1:2 (*e.g.*, red clover, Landsberg mixture, sweet lupine, etc.) requires half the above mentioned quantities of the specific preservatives.

Sugar must be diluted with water before adding. Its quantity may be doubled if desired.

Such a silage carefully prepared and fermented below 25° C. (temperature of the mass) will be satisfactory.

In order to have a comparable basis for silages of diverse nature, it is suggested that acidity be expressed in terms of percentage in the dry matter.

J.G.A.

73. **Untersuchungen über den Futterwert und die Futterwirkung eines unter Bakterienzusatz eingesäuerten Gemenges aus Erbsen, Wicken und Peluschken im Vergleich zu unbehandeltem Gärfutter gleicher Zusammensetzung. (Studies of the Feeding Value and Effect of a Mixture of Peas, Vetches and "Peluschken" Ensiled with Bacterial Cultures, in Comparison with Ordinary Ensilage of Similar Composition.)** K. Richter and J. Herbst, Institut für Fütterungstechnik der Forschungsanstalt für Tierzucht in Kraftborn (Tschechnitz), Kreis Breslan. Biedermann's Zentralblatt, Abteilung B., Tierernährung *10:* 1, 59–67, 1938.

Mixtures of the plants noted were fermented in air-tight receptacles with and without addition of bacterial preparations. Trials with ruminants (wethers) showed a higher digestibility of the N-free extract of the fodder where the bacterial preparation was added. Digestible crude protein and starch value were the same in both kinds of silage.

Later trials with nine milch cows showed that the silage fermented with the bacterial preparation was not superior to the ordinary silage either in its effect on milk yield or production of butter fat, or gain in live weight.

J.G.A.

74. Ten Years of Experimental Results on Cultivated Pastures. T. M. Olson and T. A. Evans. So. Dak. Bull. 324, 1938.

Alfalfa, sweet clover and sudan were used as pastures for dairy cows of quite uniform size and of different breeds.

Observations on bloating were made. Little trouble occurred but when bloating was observed, it took place usually in the evening and only at periods of rapid growth of the pastures.

The palatability of the plants ranked in order were, sudan, alfalfa, and sweet clover. Cows were observed to eat weeds in the sweet clover pasture until accustomed to the clover.

Milk from these cows was unaffected in flavor when they were removed from the pasture 2½ to 3 hours before milking.

Net nutrients per acre showed ranking of the plants in the order, sweet clover, alfalfa, and sudan.

Loss in weight was observed in the cows on sweet clover.

Length of pasture season was about the same for sweet clover and alfalfa but was shorter for sudan. Lack of rain shortened the period for all pastures.

Milk and butterfat produced per acre was as follows:

	Lbs. milk	Lbs. fat
Sweet clover	3668.5	146.68
Alfalfa	3307.9	135.54
Sudan	2766.5	109.95

J.C.M.

75. The Effect of Sunlight on the Growth, Production and Reproduction of Dairy Cattle. T. M. Olson. So. Dak. Bull. 319, 1938.

Two groups of Holstein calves were observed through several generations. One group and their descendants were not exposed to direct sunlight and were called the no-sunlight group. These individuals grew normally, conceived, and reproduced to the fifth generation with no apparent abnormalities. The blood calcium and phosphorus remained normal. The breaking strength of the bones of the sunlight and no-sunlight groups was normal and about the same. They did show inability to withstand the strain of producing milk and maintaining the level of production of the first generation. There was a consistent and marked decline in the butterfat production. They did, however, remain in good physical condition.

Analysis of milk of the two groups showed no significant difference. Milk from the sunlight group when fed to pigs showed it to contain appreciably greater vitamin D potency.

A part of the bulletin includes a study of the effects of winter-produced and summer-produced milkfat. The latter was definitely superior in its effects on the skeletal development of rats. The rats were fed on a rachitogenic ration (Steenbock 2965) and one group received 5 and 10 per cent levels of the milkfat produced as previously indicated. J.C.M.

DISEASES

76. Bovine Tuberculosis in Children. Anonymous. Am. Jour. Pub Health *28:* 11, 1334, 1938.

A report of work on the subject at the University of Toronto under the National Research Council of Canada is discussed. In a series of 500 tuberculous children, in 9.6 per cent of extrapulmonary tuberculosis, the bovine type was found to be the infecting organism. In every case, it was found that the tuberculous children had been fed raw milk and in three cases the disease was actually traced to the responsible animal. The present report shows that the incidence of bovine tuberculosis in children has fallen from 13.5 per cent in 1935 to 9.6 per cent at present, which is attributed both to the control of cattle tuberculosis in Ontario and to the greater use of pasteurized milk. M.W.Y.

77. Bovine Mastitis the Relation of Streptococci to Physical Changes Occurring in the Udders of Dairy Cows. W. T. MILLER and H. W. JOHNSON, Bur. of Animal Ind., U. S. Dept. of Agr., Beltsville, Md. Am. Jour. Pub. Health *28:* 10, 1222, 1938.

Composite milk samples from 629 cows in 9 dairy herds were examined for the presence of streptococci by the Hotis test, microscopic examination of incubated milk and culture of incubated milk on blood agar plates. Streptococci were detected in 298 milk samples. No streptococcus infected cows were found in 4 herds with a total of 82 animals. Seven groups of streptococci were found of which Group A predominated.

The udders of 617 cows were examined by palpation for indurations. Of the 291 cows infected with streptococci, 2.4 per cent were in Class 1 (free of indurations), while 11.6 per cent of the uninfected cows fell in this group. In Class 2 (slight indurative changes), there were 18.2 per cent of infected cows and 29.4 per cent of uninfected cows. There was no significant difference in Class 3 (definitely indurated) between the two groups, but in Class 4 (marked physical evidence of mastitis) there were 24.7 per cent infected cows against 9.2 per cent uninfected. M.W.Y.

FOOD VALUE OF DAIRY PRODUCTS

78. Nutritional Aspects of Milk. W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. Jour. Milk Tech., *1:* 3, 22–26, 1938.

In general it is almost impossible to permanently change the chemical composition of milk. The iodine content, however, can be readily changed by varying the cows' intake of this element.

All of the six generally recognized vitamins A, B, C, D, E, and G are found in milk.

Fresh milk is rather a constant source of vitamin B and E, but varies considerably for the others.

Little is known, however, of the vitamin E content of milk other than that 5 per cent of butterfat in the diet will allow normal reproduction in an experimental animal.

Milk is a fair source of vitamin B, but the amount present in milk cannot be increased by feeding methods.

G is abundant in milk, but the amount can be varied by feeding feeds richer in this vitamin. Young grass is apparently a richer source than more mature grass.

Milk varies considerably in vitamin C content. The content is more than it was formally credited with. It cannot be increased measurably by feeding. Breed and stage of lactation are important factors. Of breeds studied, Brown Swiss had highest content and Holsteins lowest. Vitamin C content is highest in fresh milk. High temperature, short-time holding method of pasteurization has little if any effect on it while low temperature long-time holding may be quite destructive.

Milk is a relatively poor source of vitamin D. Highest values in July, August, and September and lowest in February. The content can be increased by feeding.

The vitamin A content of milk is of two forms. The precursor, carotene or colored form, and the true vitamin A or colorless form. The amount of each present depends upon the feed and the breed of the cow. Deeply colored milk from a Guernsey cow would not contain any more vitamin A than the less deeply colored milk from a Holstein cow if both were fed alike. Feed is an important factor. L.H.B.

79. Present Status of Vitamin Milks. E. V. MCCOLLUM, School of Hygiene and Public Health, John Hopkins Univ., Baltimore, Md. Am. Jour. Pub. Health, *28:* 9, 1069, 1938.

The vitaminization of milk with respect to vitamin D is of established value. In the opinion of the author, milk distributors and health officials will do well to delay offering to the public any other kinds of fortified milks than vitamin D milks until the medical profession asks for them.

M.W.Y.

80. Effect of a Milk Supplement on the Physical Status of Institutional Children. 1. Growth in Height and in Weight. Lydia J. Roberts, Ruth Blair, Blanche Lenning and Marguerite Scott, Dept. of Home Economics, Univ. of Chicago, Chicago, Ill. Am. Jour. Diseases of Children, *56:* 2, 287, 1938.

The growth of 3 groups of institutional children, consisting of 36 children each, was followed throughout a calendar year. One group remained on the institutional diet as the control; one received daily an additional pint equivalent of evaporated milk and one an equal amount of irradiated evaporated milk.

By all methods of comparison the growth of the children given a supplement exceeded that of the controls. The average excess in mean gain in height was 0.3 inch for both milk groups and in weight was 2.27 pounds for the plain milk and 1.4 pounds for the irradiated milk group.

In the matter of illnesses there was little difference between the three groups and the average number of days for all illness was remarkably alike in the three groups. W.H.R.

81. Effect of a Milk Supplement on the Physical Status of Institutional Children. 2. Ossification of the Bones of the Wrist. Vera MacNair and Lydia J. Roberts, Dept. of Home Economics, Univ. of Chicago, Chicago, Ill. Am. Jour. Diseases of Children, *56:* 3, 494, 1938.

Roentgenograms of the wrists and hands of 108 children were taken at the beginning and end of a calendar year, and the progress in osseous development, as indicated by changes in the Carter ossification ratio, was followed. The grouping and dietary regimen of these children have been noted in the preceding abstract.

By all methods of comparison the groups given a supplement (irradiated or non irradiated evaporated milk) showed greater progress than the control group on the rather mediocre institutional diet. The differences were significant and tended in the same direction.

There was no significant difference between the two groups receiving milk. W.H.R.

82. Vitamin A, Carotene and Vitamin C Content of Canned Milk. O. Meulemans and J. H. DeHaas, Dept of Pediatrics, Medical College, Batavia, Netherland East Indies. Am. Jour. Diseases of Children *56:* 1, 14, 1938.

Determinations were made of three to six samples of each variety of imported canned milk obtainable in the retail trade in Batavia. The milk was diluted in the manner indicated on the label as adapted for infant feeding.

Diluted evaporated milk and sterilized whole milk were found to have the same vitamin C content as fresh cow's milk or about one third that of mother's milk.

Milk powder, sweetened canned milk, sweetened skim milk, buttermilk (either powdered or evaporated) and lactic acid milk powder after dilution contain on an average one-half the vitamin C of fresh cow's milk.

Milk powder, sweetened condensed milk, evaporated milk and sterilized milk have a carotene and vitamin A content which lies between that of stall milk and pasture milk in Europe (*i.e.*, from 10 to 25 micrograms and from 20 to 55 International units per 100 cc. of diluted milk).

Sweetened skimmed milk contains practically no vitamin A substances. Evaporated buttermilk and buttermilk powder after dilution contain as few vitamin A substances as fresh buttermilk.

The powder of lactic acid milk contains the amount of carotene and of vitamin A to be found in fresh stall or pasture milk. W.H.R.

83. Your Food and Your Future. E. V. McCollum, Johns Hopkins Univ., Baltimore, Md. Milk Dealer, *28:* 3, 72–74, 1938.

A discussion of food in regard to protein, mineral, and vitamin content. The better sources of these constituents are given and the symptoms produced by their deficiency described. C.J.B.

84. Determination of Ascorbic Acid (Vitamin C) in Milk. O. F. Garrett, New Jersey Agr. Exp. Sta., New Brunswick, N. J. Jour. Milk Tech., *1:* 3, 37–39, 1938.

This is a slight modification of the method proposed by Guthrie and Sharp, of Cornell, and is a practical and usable method intended for use in dairy and commercial laboratories.

Detailed methods for preparing reagents and procedure for making the tests are given. L.H.B.

ICE CREAM

85. The Use of Frozen Condensed Milk in Ice Cream. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *2:* 1938.

(A) H. F. Openlander and J. H. Erb, Ohio State Univ., Columbus, Ohio. p. 35.

The authors conclude that frozen condensed milk, properly frozen and stored is satisfactory for use in ice cream. The storage period studied was up to 13 weeks.

The first noticeable defect which occurred in the ice cream containing thawed frozen condensed skim milk was a curdled melting appearance. Frozen condensed skim milk testing from 27 to 30 per cent total solids was less prone to produce a curdled melting appearance in ice cream than when the total solids of the condensed product was 40 per cent. The lower and more constant the storage temperature of the frozen condensed skim milk the less trouble was experienced with the melting appearance of the ice cream. Freezing the ice cream containing frozen condensed skim milk on a continuous freezer produced an ice cream slightly more curdy than that frozen on the batch freezer. The use of 0.3 per cent sodium citrate, added to the mix at the pasteurizer corrected the curdy and wheyed off condition which might otherwise result from the use of frozen condensed skim milk in ice cream.

(B) E. L. REITCHART, Univ. of Nebr., Lincoln, Nebr p. 45.

From his experiments, the author concludes that where skim milk is available at a low cost during the early summer it might well be condensed and placed in frozen storage for later use in ice cream. When the thawed frozen cream was unusually curdy, it was made homogeneous again by homogenization of the melted product. It was found that mixes containing the frozen condensed skim milk differed but little from those made from fresh products A slight decrease in whipping ability was noted in the mixes containing the frozen products.

In a discussion of this subject following the presentation of the paper, it was stated by the author that the addition of salts to prevent a curdy melting appearance of the finished ice cream was found unnecessary. The suggestion was made from the audience that sweetening the cream before freezing had been found desirable. The author stated that the experiments with frozen condensed skim milk were being continued and the addition of sugar to the product was being studied. M.J.M.

86. Continuous Flow Mix Making. RIDGWAY KENNEDY, JR., Abbotts Dairies, Inc, Philadelphia, Pa. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *2:* 29, 1938.

A description is given of a continuous flow system for handling 2000 gallons of mix per hour. The method was designed to decrease to a minimum the physical effort necessary on the part of the operating crew and to meet all local requirements as to quality control. A diagrammatic flow sheet is given of the machinery and methods used in the mix making operations. M.J.M.

87. The Selection of Butter for Use in Ice Cream. W. B. COMBS, Univ. of Minn., St. Paul, Minn. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *2:* 22, 1938.

The types and grades of butter on the market from which the ice cream manufacturer might make a selection are presented. Data are given to show that the grade of butter used influences the consumers reaction to the finished ice cream. High scoring sweet cream unsalted butter is recommended as the type and grade best suited for use in ice cream.

Recommendations are made that the ice cream manufacturer should set up definite specifications as to type and grade, pasteurization temperature, sanitary requirements in churning, washing and working, moisture content and keeping quality of the butter he wishes to purchase for use in ice cream.

M.J.M.

88. Selection and Preparation of Fruits and Nuts for Ice Cream. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *2:* October, 1938.

(A) **Fruits.** CARL KOERVER, The Borden Co., Brooklyn, N. Y. p. 7.

A fairly complete discussion of the selection of fruits for use in ice cream is summarized by the author as follows:

To produce a strawberry pack of firm texture it is necessary that the fruit be processed immediately after receipt in the plant. The washing has to be done gently, the fruit has to be dried before going into the pack. Enough sugar has to be used to toughen the tissue. The barrels should be rocked, and the cans should be shaken or rotated. The sugar should have a chance to enter the fruit so that it is all melted before the actual freezing sets in and then the freezing should be done as quickly as possible. Based on our experience, a 2 plus 1 pack is recommended.

A properly packed barrel, especially 2 plus 1, should be of the same good quality as tins.

Thawing-out of the fruit at room temperature so that no ice crystals remain, holding slightly above freezing temperature and, when used for injectors, separating the fruit when it is still firm and cold, is absolutely necessary.

The making of a special strawberry mix is advocated to take care of the increase in sugar content due to the higher-sugared strawberries, and also to make the use of plenty of fruit, up to 35 per cent, possible without resulting in too sweet a finished product.

Fruit, such as peaches and pineapple, which are picked in an unripe stage, should be properly ripened before packing.

The use of benzoate of soda as a preservative in any pack should be avoided.

The size of the unit, barrels, half barrels, tins, cans, slabs, should depend on the quantity of fruit used at a time. No open containers should be held but the unit should be emptied.

Utensils for handling the fruit in the ice cream plant should always be washed and sterilized and kept in a clean place.

If these suggestions are followed, fruit ice cream with a clean flavor will result, it will have the same low bacterial count as vanilla ice cream and be negative in B. Coli.

(B) **Nuts.** C. A. IVERSON, Dept. of Dairy Industry Iowa State College, Ames, Iowa. p. 16.

With the advent of buttered pecan a new era in the use of nuts in ice cream began. Experiments have shown that dry roasting of nuts which are then treated with melted butter is a superior method to that of roasting the nuts in butter. A considerable amount of the flavor comes from the butter, which should be heated to about 330° F., then salted. Roughly 4 pounds of nuts is recommended for 10 gallons of finished ice cream.

Suggestions are given for preparing the following kinds of nuts for use in ice cream: Pistachio and Brazil Nuts, Filberts, Cashew Nuts, English Walnuts, Pine Nuts, Butternuts and Peanuts.

Nut meats for use in ice cream must satisfy three conditions. They must impart a distinct and pleasing flavor, must remain crisp and must be tender in the finished ice cream. The author states that it is a question to what extent nuts should be prepared in the ice cream plant for use in flavoring ice cream. He feels, however, that the freshness of the nuts is such an important consideration that some type of nut roasting equipment may be almost as necessary as mixing or freezing equipment. M.J.M.

89. **Checking Ice Cream in the Hardening Room and on Trucks.** Anonymous. Ice Cream Rev., *22:* 4, 30, 1938.

Accounting forms for a medium sized ice cream company are reproduced that will assist one in getting an accurate check on the ice cream going into the hardening room and that loaded on trucks. J.H.E.

90. **Drinking Glass Contamination and Sterilization.** RICHARD V. FELLERS, Health Officer, Nutley, N. J. Ice Cream Rev. *22:* 3, 108, 1938.

Results of a survey investigating the bacterial contamination of drinking glasses in restaurants and taverns are given. Conditions surrounding the washing of glasses were found quite unsatisfactory. Large numbers of organisms were found on the glasses of a majority of the establishments. The investigation showed that a sterilizer using sterilamps as the sterilizing agent effectively reduces the bacterial contamination of drinking glasses, and that this form of sterilization is practical and adaptable to general use. J.H.E.

91. **Frosted Foods.** LUIS GIBSON, Geo. H. Gibson Co., New York City. Ice Cream Rev., *22:* 3, 84, 1938.

Suggestions are given for popularizing frozen foods. Sales can be promoted with a consistent program of skilled store salesmanship, effective store displays, and advertising framed to appeal to the fundamental interests of the housewife. It is suggested that ice cream be sold at frosted foods outlets. J.H.E.

92. **An Antioxidant for Dairy Products.** C. D. DAHLE, Dairy Dept., Pennsylvania State College, State College, Pa. Ice Cream Trade J., *34:* 12, 16, 1938.

References are made to literature indicating that oatflour may be used to prevent the occurrence of oxidized flavor in potato chips, lard, peanuts, milk, candy, coffee, fish, and fish oils. It has been shown that 0.5 per cent oatflour prevented or delayed the oxidized flavor in strawberry and vanilla ice cream, improved the texture, and made possible a reduction in the amount of gelatine. Two per cent oatflour added to 40 per cent cream after pasteurization delayed oxidation in frozen cream. This product may be added to cream for buttermaking, for the purpose of enhancing the keeping quality of the butter.

W.H.M.

93. **More Money with Malted Milks.** Ice Cream Trade J., *34:* 12, 14, 1938.

Four fundamentals to be observed in making good malted milks are fresh, pure ingredients, used in sufficient quantities, milk that is very cold and sufficient mixing. A recognized formula for a malted milk is: 1½ oz. syrup, 2 #20 scoops ice cream, 6 oz. cold milk, and ½ oz. malted milk powder. Companion items that can be sold with malted milks are toasted raisin cake, small peanut butter sandwiches, various desserts, cinnamon toast, regular sandwiches, and various other special items. Mocha, coffee, planter's special, banana, and chocolate mint are some variations which may be used. Dealers will usually cooperate in merchandising 20 cent malted milks, and the customer will pay the price provided the malt is made of high quality ingredients.

W.H.M.

94. **Suggestions for Sanitation of Ice Cream and Ice Cream Plants.** J. H. FRANDSEN, Head, Dep't of Dairy Industry, Mass. State College, Amherst, Mass. Jour. of Milk Tech., *1:* 2, 14–17, 1938.

Besides the ingredients as sources of high bacterial counts in ice cream, the equipment used may be a factor, and also the human equation.

L.H.B.

95. **Standardization of Rulings on Equipment and Supplies.** HARRY S. CALVERT, President Dairy and Ice Cream Machinery and Supplies Ass'n, New York City. Jour. of Milk Tech., *1:* 2, 10–13, 1938.

This is an appeal to unify or standardize the rulings and regulations concerning equipment so that they would be acceptable to all authorities. This could be done by joint meetings of committees appointed from both the Milk Sanitarians and the Dairy and Ice Cream Machinery and Supplies Association.

L.H.B.

96. Maintaining Ice Cream Quality in California. F. W. MILNER, Calif. Dept. of Agriculture, Sacramento, Calif. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *2:* 60, 1938.

Although laws and regulations are primarily for the consumer's protection, it has been found that the industry itself recognizes and appreciates the value of reasonable standards. Enforcement agencies should realize that proper enforcement requires patience and good sound judgment.

In a discussion of factory sanitary requirements, and ice cream standards for food solids and bacterial content, the author presents excerpts from the California Sanitary Code for the manufacture of ice cream. The need for revision of sanitary regulations each year is set forth. The results from the enforcement of the state regulations in California is shown graphically by a comparison of bacteria counts for 1928, 1930, 1937, and 1938. In 1938 62 per cent of the ice creams tested contained less than 10,000 bacteria per cc. and only 6.5 per cent exceeded 100,000 bacteria per cc. In 1928 results for the like groupings were 18 and 70 per cent, respectively.

In California the inspection service has attempted to help those manufacturers without sufficient volume to justify the expense of proper laboratory control. A considerable amount of educational and service work has been done, especially with such groups which are unable to help themselves. Results indicate that the improvement in quality of ice cream demonstrates the value of this cooperative program. M.J.M.

97. Maintaining Ice Cream Quality in New York City. S. ABRAHAM, Division of Milk Inspection, Department of Health, New York, N. Y. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *2:* 55, 1938.

A more serious attempt to control the sanitary quality of ice cream began in 1933 with the passage of a revised set of regulations copied after those already in use for market milk. The routine control of the wholesale manufacturing establishments of ice cream consists of physical inspection of the plants and the supervision of methods of processing and handling. Equipment and containers are inspected for sanitary construction, cleanliness, and sterility and the products are sampled for chemical and bacteriological quality. Equipment must be completely dismantled after each day's run and properly sterilized both before and after use.

The bacteria counts obtained for the first 9 months of 1938 showed that 59.7 per cent of the samples of ice cream contained less than 5,000 bacteria per cc. while 9.5 per cent were in excess of the permitted maximum of 100,000 per cc.

Recognizing that the inspection personnel cannot alone insure a product of high sanitary quality, an attempt has been made to reach ice cream manufacturers and their employees as well as equipment manufacturers. Before the 1938 ice cream season reached peak production, a series of lectures were given to the ice cream manufacturers and their key employees on the subject of, "The Production of Quality Ice Cream." It was generally conceded that "quality consciousness" was created through this course. M.J.M.

98. Cold Storage Lockers. Anonymous. Ice Cream Rev., *22:* 5, 54, 1938.

The possibilities and problems involved in establishing cold storage lockers in connection with an ice cream business is discussed. The view is expressed that these establishments are destined to be an important and permanent factor in the frozen foods industry. Refrigerated lockers are rented to consumers at from $10 to $15 per year for the storage of perishable foods.

J.H.E.

99. Frosted Foods in the Retail Ice Cream Store. JOHN HUMPHRIES, Dayton, Ohio. Ice Cream Rev., *22:* 5, 52, 1938.

The experience of a chain of retail ice cream stores in handling frozen foods is given. The sales were made up of approximately 37 per cent fruits, 34 per cent vegetables, 23 per cent seafoods and 6 per cent poultry.

J.H.E.

100. Sanitation at the Soda Fountain. Anonymous. Ice Cream Rev., *22:* 5, 28, 1938.

The dangers of unclean glassware or silverware at the fountain are emphasized. Paper service is recommended as more sanitary and enables the store operator to handle the crowds with less confusion during rush periods.

J.H.E.

101. Increasing Sales of Bulk Ice Cream. Anonymous. Ice Cream Rev., *22:* 5, 22, 1938.

102. Control of B. coli in the Manufacture of Ice Cream. CHARLES PALEY, Certified Laboratories Inc., New York City. Ice Cream Rev., *22:* 4, 33, 1938.

A summary is given of the common causes for contamination of ice cream with *B. coli.* In eliminating *B. coli* greatest stress should be placed on the sterilization of the equipment coming in contact with the pasteurized product and on the quality of the ingredients added after pasteurization. J.H.E.

103. The Consumer Movement—and How to Meet It. L. O. BROWN, Assoc. Prof. of Marketing and Advertising, Northwestern University, Chicago, Ill. Ice Cream Rev., *22:* 4, 34, 1938.

The consumer movement can be an asset to business. It is essential that market research and market analysis be made in order to know what the consumer thinks. It is also important to give the consumer a better knowledge of products and services.

The results of a survey attempting to find out what the housewife thinks about ice cream are given in this article. Amongst a typical group, 49.5 per cent believed that commercial ice cream contains cornstarch as a filler. Forty-four per cent believed that packaged ice cream contains a filler not present in bulk ice cream. Fifty per cent of the women believed ice cream tends to make people fat. Other results are given. J.H.E.

104. Dipper Sinks with Running Water for Ice Cream Service. C. A. Abele, Director Div. of Inspection, Ala. State Health Dept. Ice Cream Rev., *22:* 3, 74, 1938.

A study of 40 samples of water in which ice cream dippers were immersed revealed that the bacterial content of the water ranged from 600 per cc. to 20,000,000 per cc. The average count of the water from five receptacles into which a small stream of water was constantly flowing was 9,500 per cc., the extremes being 600 and 77,000 per cc. J.H.E.

105. Special Flavors. Anonymous. Ice Cream Rev., *22:* 3, 49, 1938

During the cold months of the year when consumption of ice cream depends on taste appeal, the frequent introduction of new flavors is worth a great deal in maintaining volume. Items such as Hawaiian Fruit Salad, Pistachio nut, Banana nut and Marshmallow nut are suggested as distinctive flavors for winter sales. J.H.E.

106. Some Sanitary Problems of the Ice Cream Industry. Robert C. Hibben, Exec. Sec. of Int. Assn. of Ice Cream Mfrs., Washington, D. C. Jour. Milk Tech., *1:* 3, 43–48, 1938.

The chief sanitary problem of the ice cream industry is the lack of uniformity in regulations imposed upon the industry in the different states, counties and cities by the various health departments.

Instances are cited of duplication in inspection when products are bought or sold in different cities, counties, or states, the cost of which must be borne by the industry.

Simple regulations adequately enforced are better than complicated regulations with no enforcement.

There should be equal enforcement of regulations for all plants regardless of size. L.H.B.

107. Ice Cream Sales Index. Special Bulletin of the Int. Assn. of Ice Cream Mfrs., 1105 Barr Bldg., Washington, D. C., Nov. 1938.

An analysis is given of ice cream sales for the United States and Canada for the first 8 months of 1938. Sales in the United States decreased for the 8 month period, 4.98 per cent when compared with the like period in 1937; in Canada the loss in sales for the same period was 4.66 per cent.

The bulletin also contains the percentage of the total amount of milk produced each month and the monthly production of ice cream. The contribution of the ice cream industry towards meeting the surplus milk problem is thus graphically shown. The "ice cream expense dollar" is also analyzed with a break-down showing the natural costs by percentage as well as functional costs. M.J.M.

108. Outline of Activities for the Year 1938. Robert C. Hibben, Special Bulletin of the Int. Assn. of Ice Cream Mfrs., Washington, D. C., October, 1938.

This bulletin contains a summary of the activities for the year 1938 of the International Association of Ice Cream Manufacturers as well as that of the affiliated organization, the Ice Cream Merchandising Institute, Inc.

M.J.M.

109. Report of Simplified Practice Committee. Ridgway Kennedy, Jr., Abbotts' Dairies, Inc., Philadelphia, Pa. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *1:* 59, 1938.

In 1933 the committee submitted dimensions for an ice tray package to fit in the freezing unit of the mechanical refrigerator. This package is now widely used by the industry.

In order to further the use of the mechanical refrigerator for storing commercial ice cream, refrigerator manufacturers have been urged to provide a height of at least 4 inches in at least one of the ice tray compartments in order to accommodate the various types of ice cream containers. At least two manufacturers report that suitable space has been provided for this purpose and it is believed that others will also allow sufficient head room in these compartments for the storage of packed ice cream. M.J.M.

110. Management's Responsibility for Sound Industrial Relationships. Harold B. Bergen, McKinsey, Wellington & Co., New York, N. Y. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *1:* 38, 1938.

The author summarizes as follows the steps he believes the International Association of Ice Cream Manufacturers should take in order to facilitate sound industrial relations.

1. Appoint an industrial relations committee to study labor problems and formulate recommendations to your members.
2. Study and interpret all labor legislation affecting your industry.

3. Make a survey of existing wages and hours in the various job classifications in your industry; include information on overtime practices, vacations with pay, holidays, and time off for personal matters.

4. Collect information on current practices regarding retirement plans, death and disability benefits, and unemployment benefits and dismissal and compensation.

5. Collect information on current practices regarding employment procedures, training methods, health and safety procedures, and the like.

6. Develop material to be used in connection with the training and education of employees.

7. Collect information about the progress of unionization in your industry, collective bargaining, and labor disputes (if any).

8. Develop an industrial relations code or statement of personnel policies to be recommended to the membership.

9. Prepare informational material on the industrial relations practices of your industry to be disseminated to the public.

10. Be prepared to bargain collectively for your members on a community-wide basis.

The development of the program suggested should not prove costly. It should pay definitely in terms of dollars and cents, although it may be difficult to isolate its effects in terms of increased sales or decreased operating costs. Also, several years are likely to elapse before its full benefits are realized. But unquestionably, a sound, long-range industrial relations program will prove a profitable investment. M.J.M.

MILK

111. Use of the Lactometer. L. M. LAMPERT, Dairy Service Laboratory, Dept. of Agr., Sacramento, Calif. Milk Dealer, *28:* 3, 25, 50, 1938.

The differences in specific gravity readings made on milk by different laboratories are due to the fact that there is no standard procedure for using the lactometer. It is pointed out that the United States Bureau of Standard calibrates lactometers to be read at the top of the meniscus when floating in milk, that they have been calibrating lactometers in this manner for more than 23 years, and that the principal manufacturers of lactometers in the United States calibrate their instruments to be read to the top of the meniscus. The author summarizes his discussion as follows:

1. The lactometer reading should be made at the top of the meniscus.

2. Calibration of the lactometer may be made in milk of known specific gravity.

3. Unless the prior treatment of a sample of milk is known, an element of uncertainty is present in values of solids-not-fat derived by formula from the specific gravity.

4. With samples having their maximum specific gravity, the average of results obtained by the Babcock formula from the lactometer readings and the percentage of fat agree fairly well with the average gravimetric results.

5. With samples in which the original specific gravity has been restored by heating the average of results obtained by the formula $SNF = \frac{1}{4} + \frac{1}{5} F + 0.2$ agree well with the average gravimetric results.

6. Values on individual samples obtained by formula may vary considerably from values obtained gravimetrically. C.J.B.

112. Raw or Pasteurized Milk. Anonymous. Am. Jour. Pub. Health *28:* 12, 1424, 1938.

This is a discussion of a report by a Milk Nutrition Committee in England in which 8,435 children were divided into four groups. The children were fed a diet to which milk was compared with biscuit, also as a supplementary diet, and were studied as to increase in height and weight after having been assessed clinically as to their general condition. The general results were in favor of milk as a supplement. No consistent difference was apparent in the increments between children whose supplement was ⅔ pint of pasteurized milk and the children whose supplement was ⅔ pint of raw milk. M.W.Y.

113. Practical Experience with the Scharer Rapid Field Test for Pasteurization. D. M. Roger, Borden's Farm Products, New York, N. Y. Am. Jour. Pub. Health *28:* 11, 1325, 1938.

The author discusses a year's experience with the test in plant control in which over 18,000 tests were performed. Causes of false and erratic tests are discussed. The method is simple and the author concludes that when performed with care, its accuracy closely approaches that of the longer laboratory tests. A 30 minute incubation period aided in the detection of slightly under-pasteurized samples. M.W.Y.

114. A Critical Discussion of Some Methods and Standards for Certified Milk. J. Howard Brown, Dept. of Pathology and Bacteriology, Johns Hopkins Univ., Baltimore, Md. Am. Jour. Pub. Health, *28:* 9, 1053, 1938.

The author discusses: (1) certain features of medical milk commission control, (2) the bacteriological methods and standards for certified milk, and (3) pasteurization of certified milk. With a knowledge of the required bacterial count, the compliance rating, and the uniformity rating, one obtains more nearly complete information of the bacteriological quality of the milk than is revealed by either the arithmetical or the logarithmic average. Reasons are presented for the requirement by Methods and

Standards that when certified milk is pasteurized, it shall be pasteurized and bottled on the farm where it is produced and that the pasteurizing equipment shall not be used for the pasteurization of any other grade of milk. M.W.Y.

115. Ropy Milk—Its Causes and Prevention. J. H. Frandsen and Meyer Glickstein, Mass. State College, Amherst, Mass. Extension Leaflet 181, December, 1938.

The authors describe different causes for outbreaks of ropy milk in milk plants and on farms. A method for detection of milk that has a tendency to develop ropiness is given. Remedial measures to stop a ropy milk outbreak and rules to follow to prevent ropiness are listed. H.G.L.

116. Certain Practical Aspects of the Use of Paper Milk Containers. P. H. Tracy, Dept. of Dairy Husbandry, Univ. of Ill., Urbana, Ill. Jour. Milk Tech., *1:* 3, 40–42, 1938.

This report deals with the Pure-Pak container, formed and paraffined immediately before being filled.

Some of the points studied and reported are:

A case of 12 quarts of milk in paper bottles weighs approximately 28 pounds.

It requires 12–15 grams of paraffin to coat the containers. Paraffin did not chip off of the containers into the milk.

The amount of moisture absorbed by the walls of quart containers varied from 0.5 to 2.5 grams.

Bulging of side walls was a minor problem. There was but slight difference in the rate of temperature change of milk in paper and glass containers. Milk warmed slower in paper. Effect of sunshine on milk in paper containers was not nearly as serious as in glass bottles. Consumer preference for the paper container was shown by 221 questionnaires returned by customers on the University milk route. L.H.B.

117. Report of Committee on Dairy Farm Methods. F. D. Holford, Chairman. Jour. Milk Tech., *1:* 3, 30–33, 1938.

This report deals with three topics, the sediment in milk, mastitis in dairy cows, and sterilization of dairy utensils on the farm.

Sediment. Methods of taking sediment tests are discussed, as are sources and methods of preventing sediment. Proper methods at the source of production will reduce sediment to a minimum.

Mastitis. The effect of mastitis on the properties of milk is shown in a table. The committee suggests that the following problems should be solved.

1. How much public health significance has mastitis?
2. Has the hemolytic streptococcus (epidemicus type) in raw milk before pasteurization as much significance as typhoid organisms, tubercular organisms and other pathogens?
3. Should all cases of mastitis be considered a potential danger until the specific organisms causing the infection have been differentiated?
4. Does the abnormal milk produced by mastitis cows and included in a pasteurized supply affect its quality?
5. Should prevention be considered our first line of defense in the production of safe milk?
6. Should pasteurization be accepted as our second line of defense?
7. Is it safe to place all our confidence in the pasteurizing process?
8. Due to the magnitude of the problem, are the dairy industry and public health officials reluctant to acknowledge its importance?

Sterilization of Utensils on the Farm. Milk utensils should be cleaned at the point where the water is heated.

It is very essential to have clean surfaces where chlorine is used.

In sterilizing milking machines it is more efficient to have the chlorine solution constantly dripping through the tubes than just placing the tubes in the solution.

Some means for testing the strength of the solution should be available. Starch iodine papers have been successfully used. L.H.B.

118. The Use of Social Security Funds in Training Milk Inspectors.

C. E. WALLER, Asst. Surgeon General, U. S. Public Health Service, Washington, D. C. Jour. Milk Tech., *1:* 3, 16–21, 1938.

Social security funds are available to state health departments. The state health officer may devote to milk sanitation and to training personnel for this work any part of his allotment which he may consider proper in relation to other activities. L.H.B.

119. Observations on Problems Relating to the Paper Milk Bottle.

E. WHEATON, R. H. LUECK, and F. W. TANNER, Bacteriological Laboratories of the American Can Company, Maywood, Illinois, and the Univ. of Ill., Urbana, Ill. Jour. of Milk Tech., *1:* 3, 11–15, 1938.

The results reported in this paper are of a study made on only one type of bottle, but the authors believe their conclusions will apply to other similar type bottles. The conclusions are based on results obtained from more than four years of investigation on thousands of bottles.

Conclusions reached are as follows:

Approximately 80 per cent of all fibre milk containers are sterile. Of the remaining 20 per cent more than 90 per cent showed fewer than five organisms per bottle. Escherichia coli have been consistently absent. Bacterial types found on the plates have been white staphylococci, yellow sarcina, spore forming and non-spore forming rods. Rarely have molds been isolated.

Their data indicates the best method for making tests is to rinse the bottles with 10 ml. of sterile water and then plate the entire recoverable portion.

L.H.B.

120. Report of Committee on Methods of Improving Milk Supplies in Small Communities. Leslie C. Frank, Chairman. Jour. of Milk Tech., *1:* 3, 27–29, 1938.

Only 20 per cent of the municipalities in the 1,000 to 10,000 population group have some form of milk control.

Only 38.6 per cent of the milk sold in communities of 1,000 to 10,000 population group is pasteurized. The average state milk control staffs are too small to cope with the milk problem in all the small communities in the state. To do this adequately would require a four- or five-fold increase in the present state staffs.

From a study made by the U. S. Public Health Service it is reported that 66 per cent of all milk-borne epidemics reported during the decade from 1927–1936 occurred in communities of 10,000 population or less.

Reasons for lack of inspection in small communities are given as well as some points which the committee believe should be stressed to remedy the situation.

L.H.B.

121. Plumbing Hazards in Pasteurization Plants. W. Scott Johnson, Chief Public Health Engineer, State Board of Health of Missouri, Jefferson City, Mo. Jour. of Milk Tech., *1:* 3, 3–10, 1938.

A recent survey of the plumbing and piping systems of six large milk plants indicated the need for checking these items.

A total of 210 defects were found in these plants, ranging from 14 defects found in one plant to 61 in another. Most common defect found was type 2 with 135 and type 6 with 43.

The defects were classified into six types as follows:

Type 1. Direct pipe or equipment connection between potable water supply and sewage or other contaminated water, with or without check or manual valve between, which through excessive back pressure or negative head, or both, might result in the contamination of the potable supply with sewage or polluted water. Example—Polluted water pump directly connected to potable water supply; condensers directly connected to potable water supply and also to sewer lines; drains or overflow from potable water tanks directly connected to sewer lines; mechanical refrigerating units directly connected to potable water supply and sewer lines.

Type 2. Potable water supply inlets submerged constantly or because of direct or indirect stoppage that, due to a negative head or vacuum in the potable water supply lines, might result in the contamination of the potable water supply with sewage or polluted water through back siphonage. Example—Constantly submerged inlets which are hazardous even when the fixtures are in good operating condition such as siphon jets in water closets and urinal traps, laundry washing machines, processing tanks, water softeners, and stockwater basins. Inlets not ordinarily submerged beneath the surface of the fixture contents but which at times become submerged due to carelessness in filling or to stoppage of outlets, such as flushing rim openings in water closets, urinals, and slop sinks, lavatories, aspirators, utility room sinks, drinking fountains, bottle washers, loose hose, etc.

Type 3. Sewer lines located over pasteurizers or other milk processing equipment; floor drains located in refrigerators or other rooms where food is stored or processed and in rooms where ice is made or prepared for use.

Type 4. Water supply subject to aerial pollution. Example—Potable water supply tanks with open or loose tops or covers located on roof of building or other exposed location.

Type 5. Possible infection of persons through use. Example—Incorrectly designed drinking fountains which can be contaminated by user with possible hazard to subsequent users.

Type 6. Equipment designed to hold food during processing, surrounded by or containing pipes or jacket through which the water supply is circulated. Frequently this type of equipment is rinsed with tap water just previous to use. The danger from such connections and operation is predicted upon the water supply becoming contaminated due to other faulty plumbing and the development of breaks in the pipes or jacket so that contaminated water would leak into the food supply. Example—Certain types of milk pasteurizers, milk holding vats, milk coolers, and other equipment in which dairy products are heated or cooled or both.

Illustrations of defects and means for correcting them are given.

L.H.B.

122. Steam vs. Hot Water as a Heating Medium for Pasteurizing Milk.

R. C. Strachan, Dairy Engineer, Pfaudler Co. Milk Dealer, *28:* 2, 52–54, 1938.

R. C. Strachan, Dairy Engineer with the Pfaudler Co., points out that steam heating of glass-lined vats is actually water or vapor heating. When steam enters the jacket under pressure, it expands. As it contacts the heating surface, a condensate is formed. This evidences itself as a thin film of water running down the heating surface. It is further shown that agitation is an important factor in pasteurizing milk. That the rate of circulation must be in direct relation to the temperature of the heating medium—

the higher the temperature, the faster the circulation. The latter statement is substantiated by tests at Ohio State University and at the New York State Experimental Station. The tests at Ohio State University indicated that steam may be used as a heating medium with no detrimental results providing agitation is sufficiently great to provide rapid transfer of the heat. The following conclusions were reported at the New York station:

1. Milk was pasteurized in a glass-lined vat without imparting a cooked flavor with heating water as high as 210° F. or steam at 220° F. under pressure.

2. The temperature of the heating medium did not affect the creaming properties of the milk.

3. Momentary heating of milk to a temperature of 170° F. for one minute did not produce a cooked flavor.

4. These studies indicated that milk will withstand much more severe temperatures without developing cooked flavors than is generally supposed. Cooked flavors tend to leave milk during aging and the cardboard flavor which often develops is sometimes mistaken for the cooked flavor. C.J.B.

123. Proposed Standards for Paper Milk Containers. J. R. Sanborn, New York Agr. Exp. Sta., Geneva, N. Y. Jour. of Milk Tech., *1:* 2, 41–45, 1938.

Strict sanitary methods of manufacturing, handling, conveying and storing the paper board are important. The bacteriological content of the original container board was found to be a factor in the bacterial counts of the finished container.

Certain principles of sanitation which should be observed in the manufacture and use of paper containers are given.

It is suggested that the bacteriological standard for paper containers be the same as those suggested by the American Public Health Association for glass bottles.

The regulation pertaining to paper containers adopted by the Baltimore City Health Department is considered to be deserving of further study. Suggestions are given for amending some of the items. L.H.B.

124. Milk Plant Equipment of the Future. C. Sidney Leete, Asso. Milk Sanitarian, New York State Dept. of Health, Albany, N. Y. Jour. of Milk Tech., *1:* 2, 38–40, 1938.

The use of the phosphatase test has revealed the need of certain features in our milk plant equipment. Some of these are:

1. Automatic, rather than manual control of pasteurizers.
2. Agitation of milk during holding period.
3. Air space or foam heaters.

4. A perforating device for recording thermometer charts to prevent operators pushing charts.

Some other devices needed are constant capacity pumps for continuous flow pasteurizers, improvement in can and bottle washing machinery to insure properly sterilized containers, and the use of seamless milk cans in place of soldered cans. L.H.B.

125. Discussion of the Use of the Phosphatase Test. PAUL F. KRUEGER, Director, Bureau of Dairy Products, Chicago Board of Health, Chicago, Ill. Jour. of Milk Tech., *1:* 2, 36–37, 1938.

"After intensive study it was found that the test could be relied upon to give an accurate report of the conditions surrounding the pasteurizing process."

The test brought to light many samples of improperly pasteurized milk. With the intensive use of the test, however, the number of improperly pasteurized samples are becoming rare.

Some of the causes for improper pasteurization found by the use of this test are discussed. L.H.B.

126. The Phosphatase Test. ARNOLD B. STORRS and L. H. BURGWALD, Dept. of Dairy Tech., Ohio State Univ., Columbus, Ohio. Jour. of Milk Tech., *1:* 2, 18–35, 1938.

Gives detailed instructions for making the Kay and Graham test; also instructions for preparing the phenol standards used by Gilcrease and Davis as well as instructions for Scharer's modified tests.

In a series of tests made by the Kay and Graham method on milk pasteurized in the laboratory it was found that in using the standard of 2.3 Lovibond blue units recommended by Kay & Graham for properly pasteurized milk, that a 24-hour incubation period was satisfactory for milk pasteurized at 143° F. for 30 minutes, but if the regulation permitted milk to be pasteurized at 142° F. for 30 minutes, then a 5-hour incubation period should be used.

The test could also be applied to the high temperature short time holding systems, and a 24-hour incubation period was found to be satisfactory when milk was held at 160° F. for more than 15 seconds.

There was no relation between butterfat content of mixed herd milk, and the phosphatase content. Neither was there any relationship between the phosphatase content of the raw milk and the blue color developed in the same milk after pasteurizing.

The modifications suggested by Scharer gave promise of having considerable merit. L.H.B.

127. Sanitary Aspects of Paper Milk Containers. M. J. PRUCHA, Univ. of Ill., Urbana, Ill. Jour. of Milk Tech., *1:* 2, 4–9, 1938.

This paper is a progress report on some sanitary problems connected with the use of paper containers for fluid milk.

It gives a description of the manufacturing process.

Bacteriological examination made during the process showed that the pulp was contaminated at various stages when washed with water. It was suggested that the paper mills be inspected by milk sanitarians and the purity of the water supply checked. Health certificates should be required of all persons handling the paper in the mill, in the carton manufacturing plant and in the milk plant.

Containers that are paraffined in a factory and shipped ready to be filled must be protected from insects and dust at all times. Paraffining containers at 185° F. for 30 seconds resulted in a practically sterile container.

L.H.B.

128. Phosphatase Test for Gauging the Efficiency of Mix Pasteurization.

HARRY SCHARER, Department of Health, New York, N. Y. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *2:* 50, 1938.

Modifications of the published method for the phosphatase test are given. The modifications are necessary for successful application of the test to ice cream.

At first thought it would seem that, with the use of relatively high temperatures for mix pasteurization, the phosphatase test would be of little use. However, this is not necessarily the case. The lack of effective pasteurization occurs, often due to the total or, partial omission of the holding period or to contamination with raw products by standardization.

Interfering substances must be taken into consideration, such as vanilla powder, vanillin or coumarin, some chocolate or cocoas and some color solutions. Cooked fruit products and synthetic fruit flavorings may introduce phenolic substances. Lemon flavoring introduced a phenol while lemon juice introduced the phosphatase enzyme. One brand of cold-packed strawberries introduced both phenol and enzyme. Unroasted nut meats may also introduce the enzyme.

These interferences and the subsequent color reactions emphasize the need for careful interpretation of results and the necessity for control tests on the various ingredients.

M.J.M.

MISCELLANEOUS

129. 10 Ways to Reduce Truck Maintenance.

Anonymous. Milk Dealer, *28:* 3, 26, 38, 1938.

Reducing truck maintenance is discussed under the following headings: 1. Standardization, 2. Regular inspection, 3. Frequent tune-up, 4. Adjust trucks to job at hand, 5. Better gasoline mileage, 6. Centralize purchases,

7. Good tires and retreading, 8. Extra braces, 9. Washing and greasing, 10. Frequent painting. C.J.B.

130. What's New in Farm Science. Part 1. 55th Annual Report for year ended June 30th, 1938. Agri. Exp. Sta., Univ. of Wis., Madison, Wis. C. L. Christensen, Director. Compiled by Noble W. Clark, Associate Director, and Niemen Hoveland. Bulletin 442, November, 1938.

This bulletin briefly summarizes findings of work newly completed or in progress. The following sections are of interest to the dairy industry:

Fluid milk distribution costs. W. P. Mortenson, Lawrence Witt, and Carl Heisig. This report analyzes the records of thirteen Wisconsin dairy companies for the past six to ten years.

Large dairy plants handle nearly half Wisconsin's milk. p. 7. A. W. Colebank, and R. K. Froker, Univ. of Wisconsin, and A. C. Hoffman, U.S.D.A. Of 2,882 dairy plants operated in Wisconsin in 1935, 246 handled 48 per cent of all milk produced in the state.

Consumers buy more cheese during and after sales campaign. p. 8. Asher Hobson and Marvin A. Schaars.

Inflexibility of retail prices may discourage use of cheese. p. 12. Don Anderson. Retail prices of cheese have not consistently followed the trend of factory prices during the past twenty-five years.

Cold storage locker plants. p. 24. Marvin A. Schaars.

How accurate is the Bang's test? p. 54. B. A. Beach. In four different herds six cows were found which were carriers of the Bang's organism even though they never had reacted to the blood test. Bang's test has worked so well in such a huge majority of dairy herds that its advantages far outweigh its limitations.

Seeks new products from micro-organism. p. 61. W. R. Peterson, E. B. Fred, E. McCoy, *et al.* These studies discuss lactic acid bacteria, butyric acid anaerobes, fermentation of wood sugar liquor, products of proprionic acid bacteria, industrial production of lactic acid, enzymes of micro-organisms, physiology of anaerobic bacteria, antidermatitis factor in yeast.

Make further tests on tuberculins. p. 64. J. R. McCarter and E. G. Hastings.

Trials show allowable variations in handling Swiss cheese cultures. p. 67. H. J. Peppler, P. R. Elliker and W. C. Frazier.

Bacteriology of Brick cheese. p. 68. J. C. Garey, C. C. Schmiege, and W. C. Frazier.

Brick cheese factories need better control of starters. p. 69. W. L. Langhus, P. R. Elliker, and W. V. Price.

Splitting of Brick cheese. p. 70. F. E. Hanson, D. W. Spicer and W. V. Price.

Acidity of Brick cheese. p. 72. D. W. Spicer and W. V. Price.

Measuring salt in butter. p. 73. K. G. Weckel.

Physical properties of butter. p. 73. K. G. Weckel.

Activated flavor in milk. p. 74. J. C. Flake, K. G. Weckel, and H. C. Jackson.

Rancid flavor in dairy products. p. 75. J. C. Pfeffer, K. G. Weckel, and H. C. Jackson.

Oxidized flavor in milk traced to fat-related substances. p. 75. A. M. Swanson and H. H. Sommer.

Research improves milk irradiation methods. p. 76. H. H. Sommer, H. C. Jackson, and K. G. Weckel.

Studies on legume silage. p. 88. W. H. Peterson, C. A. Elvehjem, E. B. Hart, H. Steenbock, *et al.*

Grass juice factor. p. 91. G. O. Kohler, S. B. Randle, C. A. Elvehjem, and E. B. Hart.

Some factors affecting utilization of vitamin A. p. 92. E. J. Lease, J. G. Lease, J. H. Weber, and H. Steenbock. W.V.P.

131. What We Imagine We Know about Butter Workmanship. L. C. THOMSEN, Univ. of Wis., Madison, Wis. Nat. Butter and Cheese Jour. *29:* 23, 12, 1938.

Rules for working butter are reviewed because proper butter workmanship is more important now than it was eighteen years ago. Investigations on the influence of feed, chemical composition of butterfat, manufacturing operations, and physical state of fat in butter are reviewed briefly.

W.V.P.

132. Report of Committee on Dairy and Milk Plant Equipment. WALTER D. TIEDEMAN, Chairman. Jour. Milk Tech., *1:* 3, 34–36, 1938.

One of the important items mentioned in this report deals with milk bottle caps that cover the pouring lip.

The committee directs special attention to the need of further improvement in equipment designed for pasteurizing and handling the milk after pasteurization. L.H.B.

133. Some Highway Problems of the Dairy Industry. CHESTER GRAY, National Highway Users Conference, Washington, D. C. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *1:* 66, 1938.

The dairy industry is a heavy user of national highways. It has at least 1,270 fleets of trucks containing eight or more units carrying milk, dairy products, butter, and eggs. This figure does not begin to account for the total number of trucks employed by the industry, especially since so many trucks used for hauling milk are farmer owned.

For New York, Boston, and Philadelphia, more than 49 per cent of the milk and at least 24 per cent of the cream consumed arrives by trucks. Milk received by truck in New York City increased by 6.4 per cent in two years. There is a definite trend towards the motor vehicle for transporting dairy products.

The dairy industry should actively sponsor better highway planning and carefully follow interstate commerce laws as they affect the trucking industry. There is also a need for more uniformity in sizes and weights of motor vehicles. The writer urges a careful consideration of the fundamentals of highway transportation by the dairy industry. M.J.M.

134. **Practical Suggestions for Changes in the Social Security Act.** T. J. MAHONEY, The Borden Co., New York, N. Y. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *1:* 49, 1938.

Suggestions are made for changing state regulations drawn up under the Social Security Act. These recommendations, including the "merit rating system" for unemployment compensation, it is believed, will help both the employer and employee. M.J.M.

135. **Boilers for Dairies—Their Care and Selection.** Milk Dealer, *28:* 2, 38–39, 62–68, 1938.

A discussion of the advantages, disadvantages, and care of the four types of boilers most frequently used in the dairy industry. C.J.B.

PHYSIOLOGY

136. **Maintenance of Pregnancy by Progesterone in Rabbits Castrated on the 11th Day.** WILLARD M. ALLEN and GEORGE P. HECKEL, Department of Obstetrics and Gynecology, School of Medicine and Dentistry, Univ. of Rochester. Amer. J. Physiol. *125:* 1, 31, 1939.

Pregnancy can be maintained to term in rabbits castrated on the 11th day after mating providing 2.0 mgm. of progesterone are given daily from the 11th to 15th days inclusive and 4.0 mgm. daily from the 16th to 28th days inclusive. It is apparent that the late pregnancy changes are brought about by the combined action of progesterone (produced either in part or entirely by the ovaries) and some other hormone, probably an estrogen elaborated by the placenta. The ovary need contribute nothing other than progesterone. D.L.E.

137. **Ease of Body Heat Loss as a Basic Developmental and Functional Factor in Warm-blooded Animals.** C. A. MILLS and CORDELIA OGLE, Laboratory of Experimental Medicine, Univ. of Cincinnati. Amer. J. Physiol. *125:* 1, 36, 1939.

The body is only about 20 per cent efficient in the performance of muscular work. The remaining 80 per cent must be dissipated, since the body is very sensitive to the accumulation of waste heat. Such basic metabolic factors as growth, rate of development, and fertility are shown to be dependent on the ease with which body heat can be dissipated. No matter what the air temperature level, if the radiant heat load falling on the skin is so great as to make difficult the dissipation of this heat and that internally produced, then the body's response will be a reduction in internal combustion and a slowing down of its metabolism. The avenue of heat loss seems unimportant. Adequate loss by radiation alone provides just as effective body stimulation as does a similar net rate of loss through the usual combined avenues of conduction, convection and radiation.

It would seem advisable that standards of environmental conditions be established for laboratory animals so that results obtained in different climates and seasons can justly be compared. D.L.E.

138. The Influence of Hydrogenation and Oxidation of Fats upon Their Rate of Absorption.

Margaret House Irwin, Janet Weber, H. Steenbock and T. M. Godfrey, Dept. of Biochemistry, Univ. of Wis., Madison, Wis., and Lever Brothers Company, Cambridge, Mass. Amer. J. Physiol. *124:* 3, 800, 1938.

With rats the rate of absorption of fat was found to decrease as the melting point increased above body temperature. Variations in melting point below body temperature had no effect upon the rate of absorption.

The rate of absorption of hydrogenated cottonseed oil was found to vary inversely (from 58 to 37 per cent in four hours) with the degree of oxidation (peroxide number, 0.5 to 127.7). D.L.E.

139. Rôle of Progesterone and Other Hormones in Survival of Pseudopregnant Adrenalectomized Ferrets.

Robert Gaunt and H. W. Hayes, Departments of Biology, Washington Square College, New York University, New York City, and Princeton University, Princeton, N. J. Amer. J. Physiol. *124:* 3, 767, 1938.

Symptoms of adrenal insufficiency can be prevented in adrenalectomized ferrets by injection of crystalline progesterone or by inducing pseudopregnancy. The absence of adrenal insufficiency during pseudopregnancy is probably due to the secretion of progesterone. This indicates that the function of progesterone may be more generalized than its action on the reproductive tract alone.

No adequate explanation is available for the adverse effects of estrus and the estrogens after adrenalectomy but the suggestions that they inhibit the pituitary has not been substantiated. D.L.E.

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ABSTRACTS OF LITERATURE

BUTTER

140. Some Vital Points in the Butter Industry Figured in Formulas. E. VAILLANT. Le Lait *18:* 179, 897–919, 1938.

Formulas are given for calculating the value of butter based on a series of factors, among which are the butter fat content of the milk or cream from which the butter was made, and the by-products remaining after the buttermaking operation. A.H.J.

CHEESE

141. An Apparatus for Measuring the Elastic and Plastic Properties of Cheese Curd. G. W. SCOTT BLAIR, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res. *9:* 347–350. 1938.

An apparatus is described for measuring the elastic and plastic deformations of cheese curd under a compressive load at the time it is ready for cutting. The apparatus is considered to be more desirable than those which simply determine the force required to cut the curd since the results can be expressed in absolute (C.G.S.) units. The recoverable (elastic) deformation was found to be quite large in relation to the non-recoverable deformation. The elastic deformation is inversely proportional to shear modulus and is believed to be the best single criterion of "firmness" in deciding the optimum time to cut the curd. S.T.C.

142. The Composition of Cheese Rinds. W. L. DAVIES, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res. *9:* 342–346. 1938.

Rinds from ten samples of English, three Canadian, and five New Zealand Cheddar cheese were collected and analyzed. The rinds from three Stilton cheese were also analyzed for comparison. Rinds from aged Cheddar cheese were found to contain 15 to 20 per cent of moisture, and those from unripe cheese up to 27 per cent moisture. Stilton rinds contained an average of 17 per cent moisture. On a dry matter basis, Cheddar rinds contained less salt than the inside of the cheese. Due to increased salt concentration in the water in the outer layers there is a tendency for the salt to diffuse slowly into the inner layers of higher moisture content. There is an appreciable loss of fat from the Cheddar rinds through absorption by the bandage. The rinds were found to have a higher crude protein content, a higher calcium and phosphorus content and a higher Ca/P ratio than the interior of the cheese. S.T.C.

143. The Solubility of Cheddar Cheese Curd in Sodium Chloride Solutions. F. H. McDowall and L. A. Whelan. Dairy Research Institute (N.Z.), Palmerston North, New Zealand. J. Dairy Res. *9:* 339–341. 1938.

Measurements are reported of the solubility, in water and in sodium chloride solutions of different concentrations, of the protein of cheese at various times after manufacture. The solubility in 3 to 10 per cent sodium chloride solutions reached 100 per cent within 7 days after manufacture of the cheese, and remained at that value throughout the life of the cheese (8 months). S.T.C.

CHEMISTRY

144. The Solubility of Casein in Salts of Certain Organic Acids and Its Fractionation by Means of These Acids. R. H. McKee, Columbia Univ. and S. P. Gould, Bureau of Dairy Industry. J. Agr. Res., *57:* 125–143, July, 1938.

Solutions of sodium cymenesulphonate, potassium thiocyanate and sodium benzenesulphonate of 5 and 7.5%, 2.5 and 5%, and 5 and 10% concentrations, respectively, were used as solvents for acid-precipitated casein purified by washing with acidulated water, pure water, alcohol and finally with ether. Solubility of casein was relatively high, and increased in each case with the increased concentration of the solvent, and also with increased solution temperatures which were 15° C. (59° F.), 30° C. (86° F.) and 45° C. (113° F.). The increased solubility with increasing concentrations of solutions is considered to be one proof of the non-homogeneity of casein.

Fractionation studies with the casein mentioned, and with grain curd casein and Hammarsten casein where tried, showed that soluble and insoluble fractions could be produced at 45° C. (113° F.) by 5 and 10% sodium benzenesulphonate solution and by 5% sodium cymenesulphonate. The soluble and insoluble fractions varied in their phosphorus and nitrogen contents, phosphorus and nitrogen ratio, formaldehyde absorbing ability and in their solubility in pyridine. L.M.T.

145. A Precise Method for the Determination of Carotene in Forage. Donald W. Bolin and Assad M. Khalapur, Univ. of Idaho, Moscow, Idaho. J. Ind. & Eng. Chem., Analyt. Ed. *10:* 8, 417–18, 1938.

Peterson and Hughes' modification of Guilbert's method for extracting carotene from forage has been further modified by the authors to permit the use of definite quantities of reagents and to avoid the formation of emulsions. More carotene is recovered by the modified method and more precise results obtained. B.H.W.

146. Analysis of Sugar Mixtures Containing Dextrose, Levulose, Maltose, and Lactose. F. W. Zerban and Louis Sattler, N. Y. Sugar Trade Lab., N. Y., J. Ind. & Eng. Chem., Analyt. Ed. *10:* 12, 669–74, 1938.

In the proposed method the total reducing sugars are determined by means of Fehling solution, the monosaccharides with Steinhoff's modification of Barfoed's reagent, and levulose by the method of Jackson and Mathews. Lactose is found by oxidation to mucic acid or preferably by copper reduction after fermenting off the other sugars. Four equations are thus obtained from which the percentage of each sugar can be calculated. It has been found that both maltose and lactose have a slight reducing effect on Steinhoff's reagent as well as on Jackson and Mathews' reagent, and it is necessary to apply corresponding corrections. The quantities of dextrose, levulose and lactose found in known mixtures agree well with those taken, but the result for the maltose is less reliable because it is obtained by difference. B.H.W.

147. The Isolation of a Flavoprotein from Cow's Milk. H. S. Corran and D. E. Green, Dept. of Biochem., Cambridge, Mass. Biochem. J. *32:* 2231, 1938.

It was observed that concentrated solutions of the xanthine oxidase of milk were characterized by a pronounced orange coloration. A flavoprotein was isolated which accounted for some, though not all of this color. There are, very likely, other flavoprotein compounds in milk.

In concentrated solutions the flavoprotein is orange-red in color; dilute solutions appear brownish yellow. On addition of hyposulphite to a neutral solution of the protein, the color is bleached, and restored on shaking with air. Reoxidation is instantaneous when the hyposulphite is oxidized to sulphate.

There are three main absorption bands, with maxima at 279, 350, and 450 mu respectively. The isolation and properties of a flavoprotein and its prosthetic derivative from milk are described. K.G.W.

148. The Colorimetric Determination of pH in Milk and Whey by Means of the "Wulff" pH Tester. R. Aschaffenburg, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res. *9:* 336–338. 1938.

The pH of whey determined colorimetrically by means of the "Wulff" pH tester was found to agree within about 0.1 pH unit to the values secured potentiometrically using a glass electrode. Slightly inferior results were obtained with milk. S.T.C.

DISEASE

149. Die Frischmilchschnellagglutination zum Nachweis der Abortus-Bang-Infection. (The Use of the Fresh-milk-rapid-agglutination Test for the Diagnosis of Abortus-Bang-Infection.) DR. EHRLICH. Zschr. Infektkr. Haustiere. *52:* ($\frac{2}{3}$), 163–179.

Since attempts are now being made to control infectious abortion intensive investigations are under way to improve methods of diagnosis and to detect carriers. One of the methods suggested is to examine the milk of cows by means of the agglutination test.

The test is made on fat-free milk or on whey. The milk is diluted about ten to forty times and mixed with an equal amount of either stained or unstained abortus antigen on a glass plate and incubated for 5 minutes at room temperature. Agglutination in 10 and 20 dilutions are considered as positive.

A study was made using the rapid milk agglutination test on 1044 samples of milk and the results compared to those on the blood serum of these same animals, using the tube agglutination test. There was agreement in 73.9%, disagreement in 16.3% and doubtful tests in 9.6%. For this reason this method cannot be recommended in place of the agglutination test using blood serum. L.D.B.

150. Die Wirkung des Volkmarverfahrens und der Sufrogelbehandlung auf die Rinderbrucellose. (The Treatment of Bovine Brucillosis by the Volkmar Method and by the Use of Certain Sulphur Compounds.) H. ZELLER UND W. STOCKMAYER. Zschr. Infektkr. Haustiere. *50:* $\frac{2}{3}$, 165–179.

Some writers believe that this disease may be influenced unfavorably by certain mineral deficiencies in the soil and the usual stock-feeds. By supplying these deficiencies artifically it was believed that the constitution of the animal might be so improved as to protect it from the disease. Volkmar considered it possible to use the injection method of introducing these mineral solutions. In this study sulphur compounds were employed in comparison with other mineral mixtures.

The sulphur compound mostly studied consisted of a sterile suspension of 0.3% of finely divided sulphur in gelatin, prepared commercially in Germany.

The experimental animals were selected on the basis of the agglutination and complement-fixation tests and a clinically sound udder. The tests were conducted on 18 pregnant heifers. Six were given the Volkmar treatment, six the sulphur suspension and six held as untreated controls. The first two groups were treated for 13 days and then given 100 to 200 cc. of a Brucella culture in 900 or 800 cc. of physiological salt solution. This was a pooled culture of 10 freshly isolated strains, diluted so that each cc. contained approximately 72 million organisms. The culture was introduced into the stomach by means of a tube through the nostril. Treatments were again continued until the 24th day and culture given again. Treatment was then continued until the 78th day.

Of the Volkmar group 5 animals aborted, and the sixth calf died of pneumonia caused by the abortion organism. In the sulphur treated group five likewise aborted and one calf died of penumonia. All controls aborted and all the fetuses carried the organisms. The organisms were found in the milk of all these animals. In the Volkmar group two animals developed mastitis, as compared to three in the sulphur group and five in the control.

Antibody formation was not retarded by the mineral treatments, and in fact, the appearance of antibodies in the blood was considerably accelerated.

These results are contrary to those reported earlier on naturally infected animals treated by the Volkmar method and with the sulphur compounds above described, since early reports had been very favorable for such treatment. L.D.B.

151. Kulturelle und experimentelle Untersuchungen über die Aetiologie der Aktinomykose des Rindes. (Cultural and Experimental Studies on the Etiology of Actinomyces in Cattle.) HANS SEDLMEIER. Zschr. Infektkr. Haustiere. *50:* ⅔, 129–164.

Under the term Actinomycosis, or ray-fungus disease, are included those chronic processes which result in formation of yellowish granular pus and granulation tissue containing the ray fungus. Cattle are the most commonly infected of all domesticated animals. The disease may involve any organ but is most common in the mouth, tongue, and udder.

A variety of organisms come into question in those lesions containing pus, so that various cultural methods come into use in determining the etiology and in many instances the transmission to experimental animals is necessary. Another difficulty which is encountered in this work is that in early studies improper experimental animals were selected for study, and the fact that such a variety of names was used to describe it. With the introduction of the microscope a greater degree of accuracy was obtained.

Cultures were isolated from 59 cases of actinomyces. These were obtained from 34 infections of the tongue, 25 from soft tissue and bones, 27 cultures from the first, and 21 from the last group.

In all cases from the bones *Streptothrix israeli* was isolated, from the 27 cases of actinomycosis of the mouth parts and tongue the *Actinobacillus lignieresii* was obtained. Neither the *Bacterium pyogenes* or *Staphylococcus pyogenes* were found. Mixed infections of *Botryomyces* and *Actinomyces* were not found in the same glands as has been reported in swine.

Laboratory methods for the isolation and identification of the different organisms are given. The Actinobacillus colonies were very much alike, and the organisms were Gram-negative, short, thin rods. The growth of the Streptothrix was more vigorous, and the colonies were more dry and wrinkled. Bouillon was not clouded by either of the above organisms. The Streptothrix was usually a Gram-positive rod with thickened ends and V and L shaped forms. On glycerinated potato media longer irregular threads were formed.

Both these organisms produced tubercle-like lesions in the peritoneum of rabbits and guinea pigs.

The Actinobacillus produced a more extensive fibrinous and exudative type of peritonitis, with small multiple abscesses in the liver and lungs the size of a pea. The action of the Streptothrix in the peritoneum was less severe, and in the lung there was more of a lobular type of pneumonia.

Animals injected with the Actinobacillus became somewhat allergic to mallein but not to the extracts of the Actinobacillus when grown on glycerine broth. These organisms did not appear to produce exo-toxins. A very extensive description of the organisms and their pathological changes is included. They were negative to tuberculin. Animals treated with the Actinobacillus produced sera which were weakly positive for the glandular organisms by the complement-fixation and agglutination test.

L.D.B.

152. Zur Frage der Allergen-Auswertung. (The Question of Allergic Evaluation.) Dr. K. Beller. Zschr. Infektkr. Haustiere. *50*: 4, 336–344.

A comparison was made between tuberculin, brucellin, and mallein on 120 cattle to determine specificity of the reactions. The products were injected in 0.1 cc. doses intracutaneously and reactions recorded for seven days. A skin thickening of 3 mm. or more was considered a positive reaction; 1.5 mm. a doubtful reaction.

Cattle were selected rather than guinea pigs since they are less sensitive to allergic reactions than the latter. The skin of the guinea pig may be increased in thickness as much as 10 times, while the skin of the bovine is usually only doubled in positive reactions.

Of the cattle tested 72.5 per cent reacted to tuberculin; 11.7 per cent to brucellin; 30.0 per cent to mallein. The reactions between mallein and tuberculin were parallel in all but 3 animals, and between brucellin and tuberculin in all but 5 animals.

These cross reactions are discussed in their relation to the tuberculin test, and it was suggested that certain of the "no lesion reactors" may be due to non-specific allergic response to other infections.

In general the reactions which developed for mallein and brucellin were about parallel from day to day but not usually parellel with those produced by tuberculin. In all instances the tuberculin reaction was more pronounced and usually reached its peak after the other reactions had begun to subside. The most pronounced reaction for brucellin and mallein was on the first or second day following the injection. For tuberculin, the reaction became most pronounced between the third and seventh day. L.D.B.

153. Beitrag zur Technik der abfestuften Rohrchenagglutination. (Report on the Evaluation of the Tube Agglutination Test.) ALFRED PERZ. Zschr. Infektkr. Haustiere. *50:* 4, 313–336.

This report consists largely of a discussion of the results obtained by various persons in reading tube agglutination tests for Bang's disease. A series of tests were made and the results read by different persons. The results were then evaluated on the basis of probable error determinations.

Three methods were studied.

1. The so-called single measurement method in which various amounts of diluted serum are added to 1 cc. of antigen.
2. The chain method of dilution in which a certain amount of serum is added to the first tube of 2 cc. of antigen and 1 cc., then transferred from tube to tube containing 1 cc. of antigen.
3. A recently described method in which the three lowest dilutions were made by the first method and the remainder by the serum method. Of 150 tests set up by three persons, the second was found to be most accurate and least time consuming. L.D.B.

154. Der gelbe Galt und Versuche zur Heilung mit Entozon-Infusionen. (The Control of Bovine Mastitis by Use of Entozon.) R. HELM. Zschr. Infektkr. Haustiere. *50*: 4, 268–287.

This article is somewhat of a general discussion of the present methods of controlling this disease and the use of Entozon, an acridine dye. The animals used for study contained large numbers of streptococci and leukocytes in the milk and were considered as typical and satisfactory cows for study.

It appears that this treatment is of limited value. L.D.B.

155. Die Auswirkungen und Bekampfung der Geschlechtstrichomonaden in einer groben ostpreubischen Rinderherde. (Treatment and Control of the Trichomonads of the Genital Organs of a Herd of Cattle in East Prussia.) EULER. Zschr. Infektkr. Haustiere. *51*: 3, 203–215.

It is observed that trichomonad infections are becoming more numerous in Eastern Prussia.

The inflammatory reactions produced by these parasites are described. The use and limitations of the microscopic examination for detecting the disease are presented.

Methods of treatment for infected cows and bulls are given. Materials used in these treatments are Entozon 1–1000 to 1–1250. (Entozon is an acridine dye.) Lugol's solution and 1 per cent zinc sulphate, acetic acid and sodium carbonate, and a 1.5 per cent solution of valvanol. It is recommended that treatment be continued until microscopic examinations of the secretions are free of parasites. L.D.B.

156. Weitere Untersuchungen uber das Zellbild der Milch bei tuberkiloser Mastitis. (Mit 4 Abbidungen.) (Further Observations on the Cell Findings in Milk from Tuberculous Mastitis.) M. Ziegler. III Report. Zschr. Infektkr. Haustiere. *51*: (3), 176–185.

In the second report on this problem (Ziegler, M. and Schulze, W., Z. Fleisch-u. Milchhyg. 45, 5) concluded that the monocytes content of milk, which was described in their first report (Zeigler, M., and Bergmann, G., Z. Inf-krkh. Haustiere. 43, 211), was typical of tuberculous mastitis. The presence of the giant cell and epitheloid cell groups were of essential value as an aid to correct diagnosis. This present report is a further contribution in this field.

In the samples of milk examined, in which the giant cells or cell groups were demonstrated, they found tubercle bacilli in 36 per cent. In 218 other instances in which no tubercle bacilli could be demonstrated with the cells by microscopic methods, they were able to demonstrate them later by culture and animal inoculation.

In 226 udders examined after the cows were slaughtered, miliary tuberculosis was found 29 times, lobular infiltration 184 times, caseous mastitis 11 times, and 2 times the lesions were of mixed pathology.

They were able to demonstrate the giant cells and cell groups by microscopic study in about 50 per cent of the tuberculous animals studied.

The cell types described are the Langhan's giant cell, giant cells with nuclei irregularly distributed, epithelioidal-like cells which would probably become giant cells, and epithelioid cells in which there were but two or three nuclei. These cells were best demonstrated by low magnification.

It is believed that the cell picture may be used to advantage as an aid in the diagnosis of tuberculosis of the udder. L.D.B.

157. Beitrag zur Aetiologie der Rinderleukose in Ostpreussen. (A Report on the Etiology of Bovine Leukosis in East Prussia.). Otto Czymoch. Zschr. Infektkr. Haustiere. *52:* (⅔) 187–220.

This condition has been reported under various names such as lymphomatosis, lymphoidomatosis, lympkosarcoma, leukemia and leukosis. The disease is widespread in East Prussia and appears to be on the increase. The cause is unknown but is considered by many to be of an infectious nature and spread through soil, feed, etc.

There appears to be no seasonal variation. The greatest incidence is in animals in four to six years of age, although it is observed in some animals of two years and over 13 years of age. Investigation showed that leukosis was much more common in animals pastured on loam soil, with sandy loam next. There was very little on moor or sandy soils. There is considerably more of the disease in the northern part of the area so that the region is of some significance. The distribution among wild animals is the same as among the cattle of the region. There was no definite relation between leukosis and parasitism, abortion disease, tuberculosis, or paratuberculosis. Forcing cows for milk production did not produce the disease.

The conclusion reached from this investigation is that bovine leukosis is a disease resulting from soil deficiencies. This depends on soil moisture. In those soils in which there is high ground water the fodder contains greater amounts of silicic acid and a deficient amount of other minerals. The long continued feeding of such grasses and hay will lead to inflammation of the intestinal mucosa and later to leukosis on those animals which possess an inherited predisposition to it. The conclusion is reached following an analysis of the soils, fodder, and blood of animals in various parts of the country. Also to the fact that it was not possible to transfer the disease by artificial means. L.D.B.

158. Stephanofilariosis in Niederländisch-Ostandien and ähnliche Infektionen in anderen Ländern. (Stephanofilariosis in Dutch East Indies and Similar Infections in other Lands.) F. C. KRANEVELD. Zschr. Infektkr. Haustiere. *53*: 4, 291–332. (42 illustrations.)

This is a discussion of the skin infestation of domesticated animals, chiefly cattle, in Sumatra, Java and other East India areas.

A classification of the parasites is given. A description of the parasite and the lesions produced by their infestation is presented.

Attempts to demonstrate an insect vector failed. No treatment was found effective. L.D.B.

159. Die Feststellung der Abortus-Banginfection (Bruzellose) des Rindes mit Hilfe serologischer Verfahren. (The Detection of Brucellosis in Cattle by Means of Serological Methods.) M. SEELEMANN AND A. PFEFFER. Zschr. Infektkr. Haustiere. *53*: 3, 211–235.

In this investigation two technics were used, one using the Sachweh extract and the second the Meinicke extract. (The method of preparing these so-called extracts is not published.) These methods were used with blood serum, whole milk, and milk serum.

The technic of the test is to set up the tubes in racks and to place 1 cc. of the extract in one series of tubes and in a second series varying amounts of 5 per cent salt solution and antigen (0.02 to 0.3 cc. of antigen) to make a total volume of 10 cc. All the tubes are heated at 57° C. in water bath for a short period and the content of each pair of tubes together with 0.1 cc. of the proper dilution of the test serum added and the tubes centrifuged at 3000 r.p.m. for ten minutes. The flocculation sediment is then read. In a positive reaction the supernatant fluid is clear and the sediment collects in a button at the bottom of the tube. In negative tests the button is small and the supernatant fluid is cloudy. A similar technic is used for each type of extract except that for the Meinicke extract the tubes were centrifuged at 2000 r.p.m.

A complete flocculation occurred with a positive serum of 1–800 titre when the antigen was used in 0.1 cc. or greater amounts. When a serum of 1–6400 titre was used the same result was obtained with 0.03 cc. of antigen. The technic with whole milk and milk serum is the same as with blood serum.

It is concluded that the Meinicke extract is more satisfactory than that of Sachweh because the differences between positive and negative reactions are easier to read, although the Sachweh technic appeared to give a higher percentage of positive reactions when milk was used. In all three tests the uninfected animals gave completely negative reactions with all these methods. L.D.B.

160. Ist die Ratte an der Verbreitung des ansteckenden Verwerfens mitbeteiligt? (Is the Rat Concerned in the Distribution of Infectious Abortion?) A. SANDHOLM. Zschr. Infektkr. Haustiere. *53*: 3, 201–210.

In order to answer this question, five groups of rats were fed Brucella cultures. One group received the infectious organisms in milk for two consecutive days; a second in feces; a third received three highly virulent Brucella cultures suspended in milk; a fourth group were fed after-birth mixed with milk; a fifth group received similar material mixed with meal. The presence of an infection in the rat feces was determined by injecting them into guinea pigs. In group one, two rats developed agglutinins in 1–10 dilution in 9 and 12 days, and two others developed agglutinins in 10 and 11 days. In the guinea pigs inoculated with the feces of these animals, three developed agglutinins; one guinea pig became positive after being treated with rat feces obtained one day after it had received the contaminated milk, one guinea pig receiving feces from rats two days after feeding, and one from the rats seven days after feeding. None of the guinea pigs developed agglutinins after that date. Nothing developed in the second group of rats or in the guinea pigs which received their feces. In the group three rats, one guinea pig developed an agglutination titre following feeding of rat feces obtained the first day after the experiment started. Groups four and five were also negative.

The rat appears to be very resistant to this infection. In one instance the rat harbored the abortion bacillus in its intestine for at least two weeks so that it is a possible means of spreading the infection. L.D.B.

161. Die Rinderställe und die Verbreitung von Tuberkulose, seuchenhaftem Verkalben und gelbem Galt unter den Rinder eines sächsischen Dorfes. (The Influence of the Stable on the Dissemination of Tuberculosis, Abortion Disease and Mastitis.) H. HAUPT. Zschr. Infektkr. Haustiere. *53:* ½, 49–72.

This is something of a laboratory exercise in Veterinary Hygiene for certain students in Leipsig University. The introduction is by Haupt. The various sections are written by several others. The study was made in a small village near the city. The study included 25 stables and 280 animals, mostly cows (208). But 43.7% of these animals were entirely disease free; 6.7% were infected with abortion disease, 8.2% with mastitis, 28.3% with tuberculosis and the remainder with two or more diseases in combination.

It is concluded that the type of stall and the stable construction and management are of great importance in the dissemination of infectious disease among the animals occupying them. This is especially true in regard to the lighting arrangements. L.D.B.

162. Einfluss saurer und alkalischer Nahrung auf die Abortus-Bang-Infektion und auf Agglutininbildung. (Influence of the Use of Acid and Alkaline Feeds on Bang-infection and Development of Agglutinins.) ERICH LÜHRS. Zschr. Infektkr. Haustiere. *53:* ½, 43–48.

This investigation was undertaken because of the common practice of feeding silage during the winter season and green pasture during the summer. The former is considered to be an acid, the latter an alkaline diet. To determine the influence of feeding methods on abortion, records were taken on 1000 cows over two winters and one summer season. No influence was observed.

In a series of experiments using feeds with an average pH of 6.8, 7.3 and 7.5, there appeared to be no influence on the agglutinin titres of animals with abortion disease. L.D.B.

163. Ergebnisse der Untersuchungen über die praktische Verwendbarkeit einer Formolvakzine gegen das seuchenartige Verwenfen der Rinder. (Report on the Use of Formalized Vaccine in Control of Infectious Abortion.) FRITZ KRESS. Zschr. Infektkr. Haustiere. *52:* 4, 316–327.

The vaccine used was a fluid culture killed by formalin. Ten freshly isolated cultures were grown at 37° C. for two to four weeks. Two cc. of formalin were added to each 1000 cc. and held at room temperature for 24 hours with frequent shakings before use. The first dose was 10 cc., after four weeks a second dose of 20 cc., and twelve weeks later a third dose of 20 cc. was used, although the time between doses might be reduced. There appeared to be little response to this treatment except that a slight rise in temperature which subsided within 24 hours was sometimes observed. There was also some swelling at the point of injection after the second and third treatments. There was little disturbance of the milk supply. The agglutination titre of the blood increased after each treatment but disappeared before the next treatment. About eight weeks after the last treatment it was slight in 1 to 50 but negative in 1 to 12.5 after 12 weeks. The milk did not exhibit agglutinins due to the treatment.

Among 1633 animals with early infection there were 39.8% reactors and 19.3 abortions. During the first period of pregnancy after the treatments, these abortions had been reduced to 0.9%. Among 762 animals with standing infection, there were 23.2% reactors and 15.9% abortions. After treatments the abortions had been reduced to 0.6%.

Emphasis is placed on the necessity of careful hygienic measures during the vaccination period. L.D.B.

164. Ueber eine Bartonella-Infektion beim Rinde. (A Bartonella Infection in the Ox.) O. Nieschulz. Zschr. Infektkr. Haustiere. *53*: 3, 175–179.

During an examination of the blood of a calf which had been inoculated with blood originally obtained in North Africa there was observed, besides anaplasma and iroplasma, a coccoid-like parasite situated within the red corpuscles. This was arranged 4 or 5 in a chain or in a mass of ten or more. The parasites may be arranged around the periphery of the cell. They were coccoid in form, 0.2 to 1.5 microns in diameter. These forms on the periphery are more darkly stained than those within the cell protoplasm. The parasite was very similar in appearance to *Bartonella canis* and may belong to the same genus, although it was finally identified with *Eperythrozoon wenyoni.* L.D.B.

165. Die Wichtigsten sudafrikanischen Giftpflanzen. (Important South African Poisonous Plants.) Douw G. Steyn, Zschr. Infektr. Haustiere. *53*: 4, 233–349 (15 illustrations).

In the South African Union there are more than 200 poisonous plants. Several of these plants are described in this paper.

The animals commonly poisoned, the clinical symptoms and some pathological findings are given.

Plants causing photosensitization in animals with non-pigmented skins are listed with a description of symptoms which develop when these animals are exposed to sunlight. L.D.B.

FEEDS AND FEEDING

166. Digestible Nutrients and Metabolizable Energy in Certain Silages, Hays and Mixed Rations.

F. W. CHRISTENSEN AND T. H. HOPPER, North Dakota Agr. Exper. Sta., J. Agr. Res., *57*: 477–512, October, 1938.

Digestion trials with six sheep (grade wethers) during 1923, with four steers during 1923–24, and with four steers in 1924–25 are reported. Roughages studied included sweet clover-oat straw silage (bud stage), sweet clover silage (bud stage), sweet clover silage (early bloom stage), sweet clover silage (full bloom stage), sunflower silage, corn silage, sweet clover hay and stems and alfalfa hay. Concentrates included linseed meal, barley and wild oats (removed from wheat for milling). Extensive data from balance trials with these feeds are given. Chemical compositions of the feeds are given.

The silages studied were generally higher in ash, crude protein, crude fiber, and ether extract, but lower in nitrogen-free extract than the crops when ensiled. On a dry-matter basis, the sweet clover silages and hays resembled alfalfa hay in percentage of digestibility, digestible nutrients, and metabolizable energy although the sweet clover silages averaged slightly higher in digestible crude protein and total digestible nutrients. Mixing 1 part of dry oat straw with 5.5 parts of green sweet clover reduced the digestible crude protein 56% and total digestible nutrients 37% in the silage, on a dry basis as compared to straight sweet clover silage from the same crop. The sunflower silages were less palatable than the sweet clover silages, which, in turn, were less palatable than the corn silage.

On a dry basis the average percentages of digestible protein and total digestible nutrients respectively for several of the feeds were: for the straight sweet clover silage, 16.2 and 56.8; for the corn silage, 5.3 and 69.7; for the sunflower silage, 4.8 and 47.7; for the linseed meal, 32.9 and 75.8: and for the wild oats, 9.6 and 61.6.

The metabolizable energy per pound of digestible organic matter in the different feeds and rations was fairly uniform. The average of 25 individual determinations on roughages was 1.737 therms per pound and 1.767 therms for 21 mixed rations. In 25 individual trials the average metabolizable energy per pound of total digestible nutrients was 1.670 therms, and in 21 trials of mixed rations, it was 1.689. L.M.T.

167. A Comparison of the Vitamin D Potency of the Stemmy and Leafy Portions of Alfalfa Hay.

G. C. WALLIS, South Dakota Agr. Exper. Sta., J. Agr. Res., *57*: 393–396, Sept., 1938.

The leaves and stems of good quality, green-colored alfalfa hay, carefully separated, were found by the standard line-test technique, to contain approximately 10.45 and 1.72 International Units of vitamin D per gram, respectively. The leaves represented 49.4% of the sample and the stems 50.6%. L.M.T.

168. The Carotene Content of Market Hays and Corn Silage. H. G. Wiseman, E. A. Kane, L. A. Shinn and C. A. Cary. Bureau of Dairy Industry, U.S.D.A., Washington, D. C. J. Agr. Res., *57:* 635–670. Nov., 1938.

A major portion of this paper describes the methods for the determination of carotene used in the laboratory of the Division of Nutrition and Physiology at Beltsville, Md., and presents data to show the accuracy of results obtained by these methods. The largest error was found to result from the inclusion of pigmented impurities other than xanthophyll in the final carotene extracts. However, it is shown that the methods yield a useful index of the vitamin A values of feeds for cattle.

The carotene contents of a number of roughages and concentrates were determined in addition to those for corn silages and various grades of market hays. Green, growing bluegrass and alfalfa averaged 567 and 332 mg. per kilogram, respectively, on a dry weight basis. On the basis of weight as fed the following average values per kilogram were found: artificially dried alfalfa leaf meal, 151 mg.; corn silage, 14 mg.; alfalfa hay, U. S. No. 1 in color, 43 mg.; alfalfa hay, U. S. No. 2 in color, 15 mg.; alfalfa hay, U. S. No. 3 in color, 3 mg.; timothy hay, U. S. No. 1 in color, 21 mg.; timothy hay, U. S. No. 2 in color, 9 mg.; timothy hay, U. S. No. 3 in color, 5 mg.; yellow garden carrots, 91 mg.; dry corn stover, 4 mg.; and clover hay, U. S. No. 1, 23 mg. Considerable variation occurred with individual samples, averages for which are shown here. L.M.T.

169. The Level of Inorganic Phosphorus in the Blood of Dairy Cattle. S. R. Johnson, Inst. of Nutrition, Pa. State College, J. of Nutrit. *17*: 15–21, 1939.

The purposes of this investigation were to establish standard normal values for the inorganic phosphorus of whole blood of dairy cattle, and to study the variations resulting from differences in conditions as to breed, age, milk production and reproductive status. Inorganic phosphorus was determined in the whole blood of more than 200 normal cattle.

The values for calves from one to seven months averaged 6.42 mgm. per cent. There was a gradual decrease with age. Mature cows averaged 4.29 mgm. per cent. Breed of cow, the stage of lactation or the kind of roughage consumed did not affect the inorganic phosphorus content of whole blood. C.F.H.

170. Evaluation of the Vitamin A Potency of Feeds. G. S. Fraps, Texas A. & M. College, College Station, Texas. J. Ind. & Eng. Chem., Analyt. Ed. *10*: 9, 525–27, 1938.

The author discusses the importance of making vitamin A determinations in feed and reviews the methods available for the estimation of carotene therein. B.H.W.

171. Additional Nutritional Factors Required by the Rat. J. J. OLESON, H. R. BIRD, C. A. ELVEHJEM, AND E. B. HART, J. Biol. Chem. *127*: 23, 1939.

Four different deficiency symptoms have been produced in rats on synthetic diets low in the vitamin B complex but adequate in thiamine, riboflavin, choline and nicotinic acid. Crystalline B_6 was very active in preventing acrodynia, but inactive in the preventing of paralysis, hemorrhage disease, or spectacled eye condition. The relation of these deficiencies to known members of the B complex is discussed. The importance of dietary fat and carbohydrate in relation to growth was discussed. K.G.W.

172. Carotene, Isolation, and Detection of Carotene, and the Carotenes of Carrot Roots and Butter. HAROLD H. STRAIN, Carnegie Institution of Washington, Div. of Plant Biology, Stanford Univ., Palo Alto, Calif. J. Biol. Chem. *127*: 191, 1939.

Since the carotenes of butter vary with those ingested by the animals, investigations of the butter pigments must be supplemented by investigation of the carotene of the rations. Occurrence of only B carotene in butter after cows had received principally this pigment in the rations indicate that carotenes are not interconvertible in the cow. The proportions of the carotene isomers isolated from butter after cows had been fed carrots were similar to the proportions of the carotenes in the ration. The rapidity with which the carotenes of carrot roots are transferred to butter suggests that carrots may be used as a source of vitamin A in milk and butter in northern and arid regions where green fodder is not available.

Carrots contain considerable quantities of a carotene pigment the spectral absorption properties of which are similar to those of the flavoxanthins. This pigment does not occur in appreciable amounts in the leaves. K.G.W.

173. The Effect of Different Methods of Drying on the Biological Value and the Digestibility of the Proteins and on the Carotene Content of Grass. STEPHAN BARTLETT, K. M. HENRY, S. K. KON, L. W. OSBORNE, S. Y. THOMPSON AND JOSEPH TINSLEY, Nat. Inst. Res. in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. Biochem. J. *32*: 2024, 1938.

One of the outstanding features claimed for modern methods of artificial grass drying is that they involve but little destruction of the nutritive properties of the grass. The high temperatures attained during the process, however, raise the question of heat injury to the proteins. It is well known that heating lowers the biological value of the nitrogen of various foodstuffs.

Nitrogen balance experiments with rats were carried out using grass dried by artificial means and also made into hay the normal way and the effect of the various procedures on the carotene content and on the biological value of the proteins of grass were measured.

The biological value and true digestibility of the proteins of grass artificially dried at the normal running temperature (300° F.) in a rotating drum type drier were compared on rats with those of grass dried at low temperature (170° F) and those of sun cured hay. All three samples were made from one bulk of fresh grass.

The following figures were found for biological value and true digestibility; artificially dried grass, high temperature, 67 and 66; artificially dried grass, low temperature, 62 and 65; hay, 52 and 64.

The differences in biological values of the proteins between artificially dried samples and hay were statistically highly significant. Other differences were not.

The hay cured under natural conditions contained only about one-quarter of the carotene originally present in the fresh grass. Artificial drying at normal temperature preserved nearly 90% of the carotene. K.G.W.

174. Relation of Roughage to the Fat Content of Milk. E. B. Powell, Ralston Purina Company, St. Louis, Mo. Cert. Milk, *13*: 152, 9, 1938.

It was found that some long range fat variations are related to the physical characteristics of the ration. The physical condition of the roughage when it is consumed influences the fat content of the milk. The author is of the opinion that there is a definite correlation between the activities of the rumen and the composition of the milk which a cow produces. W.S.M.

175. Urea as a Protein Substitute in the Diet of Young Cattle. S. Bartlett and A. G. Cotton, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res. *9*: 263–272. 1938.

Twenty-one dairy heifers aged 7 to 17 months were used in a 42 day feeding experiment in which part of the animals received a normal-protein ration, part a low-protein ration, part the low-protein ration supplemented with urea in sufficient quantity (0.127 pounds per animal per day) to supply the same total nitrogen as the normal-protein nitrogen, and part the low-protein ration in which a small quantity of starchy food was replaced by ground-nut protein, making the total nitrogen content equal to the normal-protein ration.

Animals on the urea supplemented ration had an average gain in liveweight of 0.24 pounds per day more than those on the low-protein diet. This increase was determined to be statistically significant; therefore, the authors conclude that the animals appear to have utilized the urea in their metabolism. The animals receiving the same quantity of additional nitrogen in the form of protein showed slightly better live-weight gains than those receiving urea. S.T.C.

176. **What Is the Truth about Grass Silage?** J. W. BARTLETT, N. J. Agr. Exper. Sta., New Brunswick, N. J. Cert. Milk, *13*: 149, 9, 1938.

It is suggested that the value of grass silage be expressed as "nutrients per acre" and not just tons per acre. W.S.M.

177. **"Canning" of Legumes and Grasses.** L. J. TOMPKINS, Sheffield Farms Co., New York City. Cert. Milk, *13*: 152, 7, 1938.

A discussion of the following principal methods of curing forage: (1) natural or sun curing; (2) artificial dehydration; (3) silage green crops; (a) by natural fermentation, (b) by addition of acids, (c) by addition of molasses. W.S.M.

FOOD VALUE OF DAIRY PRODUCTS

178. **The Biological Value of Carotene in Various Fats.** E. J. LEASE, J. G. LEASE, H. STEENBOCK, AND C. A. BAUMANN, Dept. of Biochemistry, Univ. of Wis. J. of Nutrit. *17:* 91–102, 1939.

The biological response of rats to carotene administered in various oils, using the growth, cure of ophthalmia and the storage of vitamin A in the liver as biological criteria was studied. When excessive amounts of carotene or vitamin A were fed in lard, soybean oil, cottonseed oil, devitamized butterfat and a hydrogenated vegetable fat, there was no marked difference in storage. One microgram of carotene in cottonseed oil, soybean oil, decolorized butterfat, coconut oil or crude peanut oil gave approximately equal growth responses. Inferior growth was obtained on triolein, linseed oil and "refined" peanut oil. The rate of cure of ophthalmia was essentially the same for all oils. C.F.H.

179. **Planning the Best Diet for the Family.** E. V. McCOLLUM. Milk Plant Monthly *27*: 12, 23, 1938.

The author points out that good nutrition is easily attainable in this country. A wider knowledge of the way to plan the diet, and an appreciation of the importance of good food in relation to health is essential.

A diet consisting of white bread, refined cereals, excessive sugar and sweet foods together with potatoes and lean meat will not be adequate for the maintenance of health. A proper amount of protective foods when included in the daily menus will supply everything that is needed for the promotion of health.

The daily food must supply the proper kinds and amount of proteins, minerals, and vitamins.

The value, necessity and source of vitamins A, B, C, and D are discussed.

Each member of the family should have daily a quart of milk or its equivalent, two salads, two servings of cooked leafy vegetables, one serving of meat, fish or poultry, at least four eggs a week, and some fresh fruit or fruit juice. G.M.T.

180. Assays of Riboflavin and the Filtrate Factor in Certain Milk Products. T. H. JUKES AND G. A. RICHARDSON, California Agr. Exp. Sta., Davis, Calif. J. Agr. Res., *57*: 603–610, Oct. 1938.

In November, 1935, and again in May, 1936, a large amount of milk produced in the same territory, but not necessarily from the same herds, was divided and manufactured into the various dried products enumerated below. Results of chemical analyses of these products are given. Assays of the riboflavin and filtrate factors of each of the products were made, using chicks 6 to 8 days old as the experimental animals. The assays did not show any differences between the November and the May samples. The descending order of potency in filtrate factor for the November samples was: rennet whey, sulphuric acid casein whey, lactic acid casein whey, hydrochloric acid casein whey, buttermilk, skimmilk and whole milk. The order for the May samples was the same except that buttermilk was slightly superior to hydrochloric acid casein whey. In round numbers the dried wheys of various types furnished 3 to 6 units of filtrate factor per gram; dried buttermilk, 3 to 4; dried units of filtrate factor per gram; dried buttermilk, 3 to 4; dried skimmilk, 2 to 3, and dried whole milk, 1 to 2. The dried products furnished two to five times as much riboflavin as filtrate factor. L.M.T.

181. The Relation of Color and Carotene Content of Roughage in the Dairy Ration to the Color, Carotene Content, and Vitamin A Activity of Butter Fat. R. E. HODGSON, J. C. KNOTT, AND H. K. MURER, Washington Agr. Exp. Sta., and R. R. GRAVES, Bureau of Dairy Industry. J. Agr. Res., *57*: 513–529, Oct., 1938.

Three groups of 10 Holstein-Friesian cows each were winter-fed respectively on field-cured hay from perennial grass and clover mixtures, on silage made from the grass and clover mixtures and silage made from oats and peas, and on a combination of the hay and silage. All the cows were maintained on pasture alone during the summer. The color (green) and the carotene content of the roughages were determined and compared with the color (yellow), carotene content and vitamin A activity of the butterfat produced. Vitamin A determinations were made by a modification of the Sherman method. Results show that the hay was the poorest source of vitamin A in the butterfat among these roughages and grazed grass the best, while the silages were nearly as good sources as the grass. There was neither a consistent relationship between the carotene content of the feed and that of the butterfat produced from it nor between the carotene content of the butterfat and its color. However, average values showed an increase in the color of the butterfat as the carotene content increased. The carotene in the butterfat accounted for only 11.2 to 13.7% of its vitamin A activity according to calculations by the authors. L.M.T.

182. The Influence of Various Fodder Supplements on the Production and Nutritive Value of Winter Milk.

S. Bartlett, A. G. Cotton, K. M. Henry, and S. K. Kon, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res., *9*: 273–309, 1938.

An experiment is described in which comparisons were made between the effect of (a) mangels (control feeding), (b) artificially dried grass, (c) sprouted maize, and (d) kale used as dietary supplements on the production and nutritive properties of milk of dairy cows. No significant differences were found between any of the foods in their effect on yield of milk, or its content of fat or solids-not-fat. The vitamin A and carotene contents of the rendered butterfat were measured spectrophotometrically and with the Lovibond tintometer. The feeding of kale and artificially dried grass markedly increased the color and vitamin A content of the milk, but sprouted maize had no more effect in this respect than mangels.

The vitamin B complex level of the milk was not measurably influenced by the feeding of any of the fodders, but the vitamin B_2 (complex) level and vitamin C level was lowest in the milk from the cows fed sprouted maize. The growth-promoting properties of the milk produced on mangels, artificially dried grass and sprouted maize were compared. The milks were fed, supplemented either with minerals alone or with minerals plus sugar as a source of energy. In one series of experiments the milks were given ad libitum, in another the intake was "equalized." No differences were found and the authors concluded that under English conditions this technique does not disclose the presence in milk of a new essential factor. S.T.C.

183. The Vitamin A Value of Colostrum and Milk of Four Cows Determined by the Single-feeding Method.

M. M. Kramer, Margaret D. Blair, and W. H. Riddell, Kansas Agr. Exp. Sta. J. Agr. Res., *56*: 227–232, Feb., 1938.

Total vitamin A values, found by the single-feeding biological method of Sherman, were determined for the colostrum milk of two cows on the regular dairy herd ration and for two cows on the same ration supplemented with rye pasture. The milks of the second day of lactation were highest in vitamin A value, 25 and 28 International Units per gram for the cows on rye pasture and 16 and 20 units for the others. The vitamin A value was considerably reduced by the fourth day and from the seventh day until the end of the month of testing the value remained fairly constant for the four cows at approximately 2 International Units per gram. Comparisons of the total production of vitamin A per day proved highest on the first or second day of lactation. One of the cows receiving no supplement maintained the highest level of vitamin A output during the latter part of the month. In general the total daily production of vitamin A was quite constant from the end of the first week until the end of the month. L.M.T.

184. Vitamin G (Riboflavin) Content of Colostrum and Milk of Cows Determined Biologically. M. M. Kramer, Bernice L. Junnerth, and W. H. Riddell, Kansas Agric. Exper. Sta. J. Agr. Res., *56*: 233–237, 1938.

Twenty-four hour composite samples of colostrum milk from two Jersey and two Holstein cows on the first, fifth and thirtieth days of lactation, together with a sample of mixed herd milk, were tested for vitamin G by biological assay. Flavin reference curves were used to estimate the vitamin G content per gram. First day colostrum was richest in vitamin G and was estimated to contain 4.2 to 6.7 micrograms per gram. A gram of colostrum, 2 grams of fifth-day milk, and 3 grams of thirtieth-day milk gave approximately the same gains in weight for the groups of rats fed. Herd milk was estimated to contain about 2 micrograms per gram. The largest total daily outputs occurred on the fifth day, but these totals approached the yields for the thirtieth day. L.M.T.

185. Improving the Curd Size of Certified Milk. I. J. Wolman, Univ. of Pennsylvania School of Medicine, Philadelphia, Pa. Cert. Milk, *13*: 150, 5, 1938.

A general discussion of methods for producing artificial soft curd milk. The nutritive benefit of adequately homogenized milk, whether pressure treated or sonized, seems to rest in the small curds that form in the stomach. Other advantages of homogenization are: improved flavor, uniform distribution of fat, and protection against theft of cream content.

The author suggests that certified milk producers check the curd forming qualities of their product at regular intervals and report these findings to the Medical Milk Commissions. W.S.M.

186. New Deal in Nutrition Necessary for Recovery. J. A. Tobey, American Institute of Baking, New York City. Cert. Milk, *13*: 150, 7, 1938.

An adequate diet is defined. The present American diet is discussed and an improved national diet is suggested. W.S.M.

187. Vitamin C Content of Certified and Other Grades of Market Milk. C. C. DAVIS, The Medical Milk Commission of the New Orleans Pure Milk Society, New Orleans, La. Cert. Milk, *13*: 152, 5, 1938.

Using the rapid titration method as described by Sharp for determining vitamin C content of milk, data from a study of certified and other grades of market milk are presented.

The average value found in certified raw milk was about one-fourth higher than in Grade A raw, nearly twice as high as in special Grade A pasteurized, and five and one-half times as high as in regular Grade A pasteurized milk.

Bright daylight and especially direct sunlight was found to be very destructive to vitamin C in milk, even when exposed but a short time.

Fresh milk stored in a refrigerator lost about 45 per cent of this factor in 72 hours. W.S.M.

ICE CREAM

188. Consumer Preference for Ice Cream. H. E. ROBERTS AND R. B. STOLTZ, Ohio State Univ., Columbus, Ohio. Ice Cream Rev., *22*: 6, 40, 1939.

The results of a consumer quantity preference experiment in which people were allowed to eat all the ice cream they wanted are given. The results were checked against the questionnaire and sample method. In general it was found, the two methods gave similar results; however, there were some differences. For example, by the questionnaire and sample method, consumers expressed a preference for a high serum solids ice cream, but when allowed to eat all the ice cream they wanted, as was done under the quantity test, consumers ate more of the lower serum solids ice cream. Other variants so studied were color, per cent of sugar, egg yolk, per cent butterfat, type of vanilla and kind of sugar. Questionnaire and sample results also are given for consumer preference of mixes containing noticeable "off" flavors. J.H.E.

189. Low-Lactose Milk Solids in Ice Cream. W. J. CORBETT, Univ. of Ill., Urbana, Ill. Ice Cream Rev. *22*: 6, 34, 1939.

A procedure is given for the preparation of low-lactose milk solids taking advantage of the wheying off obtained when a derivative of the Carob bean is added to milk. The use of 3.5 to 4.5 per cent of the low-lactose solids in addition to 8.5 to 9.5 per cent of regular serum solids produced an improvement in the body of ice cream frozen in a batch freezer. Replacing one-fourth of the sucrose in low-lactose mixes with dextrose is recommended to prevent the ice cream from having a dry body. J.H.E.

190. Keeping Store Operations Under Control. VERNE RIGHTER, Barber Bros. Co., Chicago, Ill., Ice Cream Rev., *22*: 6, 37, 1939.

In the operation of retail ice cream stores, careful observation of the money handling, frequent checking of the register, taking inventory, and figuring dippage, alternating the employees, and a knowledge of the environment of each clerk, are recommended to prevent losses. J.H.E.

191. Colors and Flavors. D. LEVOWITZ, New Jersey Dairy Laboratories. Ice Cream Field, *32*: 10, 95, 96, 98, 1938.

Directions are given to aid in the preparation and care of colors and flavors for use in ice cream.

The following points are emphasized:

1. A perfect mix may be ruined by materials added at the freezer.

2. Colors and flavors, fruits and nuts should be selected carefully and should be stored and handled in a sanitary manner.

3. Unless controlled, materials added at the freezer can nullify the protective steps in processing the mix. W.C.C.

192. Refrigeration Problems. A. M. SCHMITZ, Servel Inc. Ice Cream Field, *32*: 10, 94, 99, 1938.

Brief instructions are given as to seasonal adjustments in ice cream cabinets, also as to means of overcoming the difficulty of Freon compressors on ice cream cabinets pumping oil out of the crankcases, thereby resulting in damage to the bearings and moving parts. W.C.C.

193. Egg Yolk Helps Sales. J. R. TURNER, Armor & Co. Ice Cream Field, *32*: 10, 29, & 30, 1938.

The author refers to a survey by Tracy and Smith of the University of Illinois in which it is claimed that the majority of the ice cream eaters prefer ice cream with high serum solids and medium fat contents. They prefer ice cream containing a stabilizer and also ice cream that is medium in color.

The author makes the following claims for egg yolk as an ingredient in ice cream.

1. The emulsifying properties of egg yolk produce small uniform air cells.

2. Egg yolk makes possible an increase in serum solids without causing sandiness. (Note: To the knowledge of the reviewer this point has not been established.)

3. Egg yolk gives ice cream a natural rich appetizing color.

4. Egg yolk permits a reduction in butterfat and improves the flavor without sacrificing sales appeal.

5. Use of egg yolk in ice cream permits a reduction in the amount of stabilizers to a satisfactory basis without a loss in body and texture.

The author claims that many people are wrong in assuming that the principal advantage of egg yolk in ice cream is improved whipping ability of the mix. He claims further that egg yolk powder is more convenient to use than other forms of egg products. W.C.C.

194. Refrigeration Problems. A. M. SCHMITZ, Servel Inc. Ice Cream Field, *32:* (12) 14, 16, 17, 18, 1938.

Eight standards are given for rating condensing units which were adopted in 1937 by the American Society of Refrigeration Engineers, Refrigeration Division of the National Electric Manufacturers Association and the Refrigerating Machinery Association. The author points out that unless the suction vapor is superheated to the same temperature used by the manufacturer in rating the unit, actual capacity may deviate from the rated capacity by as much as 15 per cent. Examples are used to illustrate this point.

The functions of a compressor and evaporator are briefly described and the advantages of using a heat interchanger are also indicated. The heat interchanger is a device consisting of two copper tubes and necessary fittings so arranged that the suction vapor from the evaporator flows through the inside tube and the warm liquid refrigerant from the receiving flows in the opposite direction in the space between the two tubes. This serves to transfer some of the heat from the liquid refrigerant to the cold suction vapor. This results in a silght increase in the condensing capacity as well as a saving in the amount of liquid refrigerant which is evaporated to cool the liquid to the evaporator temperature. In a specific example the author shows an 11 per cent saving as a result of the use of the interchanger. W.C.C.

MILK

195. Manual for Milk and Cream Testers in Maryland. C. W. ENGLAND, Maryland Agricultural Experiment Station, Bulletin 423, December, 1938.

This bulletin is a revision of Parts I and III only, of the Maryland Agricultural Experiment Station Bulletin 401. It describes practical and approved methods of testing fresh milk, preserved composite samples of milk and cream by the Babcock Method, and is designed to aid those persons desiring to pass examinations for Babcock tester's licenses on milk and cream. It also contains specifications for Babcock glassware and the Dairy Inspection Law of Maryland. C.W.E.

196. Die Bekampfung der Abortus-Bang-Infektion, der Tuberkulose und des Gelben Galtes im Plane der Mabnahmen der Rohmilchversorgung. (Control of Abortion Disease, Tuberculosis, and Mastitis in Order to Protect the Raw Milk Supply.) DR. PLATE. Zschr. Infektkr. Haustiere. *50*: 4, 287–303.

A general discussion of the milk supply of the city of Bremen. An improvement in health condition, and decrease in infant mortality, followed the elimination of the above disease from the dairy herds of that city. There is a general discussion on methods of control of those infections, and their importance as diseases in man. L.D.B.

197. The "Lactotest" of Koschuscharoff. A More Simple Method of Determining the Addition of Cow's Milk to Woman's Milk.

MARIE-ELISE KAYSER. Regional Clinic for Women at Erfurt. Le Lait *18*: 179, 930–934, 1938.

By means of a specially prepared serum called "lactotest," it was possible to detect the addition of relatively small amounts of cow's milk to human mother's milk. The mother's milk to be tested is first centrifuged to remove fat. A drop of the defatted milk is then mixed with 4 times its weight of the serum on a microscope slide. With pure mother's milk, the drop appears uniformly turbid under the microscope with a whitish cloud at the center of the drop. When cow's milk is mixed with mother's milk, a positive reaction is given after ½ minute. The liquid which was turbid at the beginning became clear, especially the peripheral liquid, with a definite coagulation. This method was considered superior to several colorimetric methods that were tested. It did not require a control, slight adulterations of mother's milk with cow's milk could be detected, expensive reagents were not required, and the reaction was rapid. A.H.J.

198. The Hygiene of Milk in Canada.

E. U. ALLARD, Chief Inspector of Milk, Quebec, Canada. Le Lait *18*: 179, 919–930, 1938.

The advantages of pasteurized milk are emphasized. Raw milk is usually much more variable in composition than pasteurized milk. Of more than 3000 samples of raw milk tested, about 26 per cent were below the legal standard of 3.25 per cent fat, while for pasteurized milk only 3 per cent were below the legal fat requirement. The reason for the variation is due to the large amounts of mixed milk that go into pasteurized lots of milk while raw milks are in many cases only the mixed milks from single farms. A.H.J.

199. Treatment and Transformation of Milk, Improvement in Quality.

W. CLAUSS, Agronomic Engineer, Berlin, Germany. Le Lait, *18*: 179, 982–999, November, 1938.

Methods of handling milk on the farm and processing it in the dairy so that better market milk, butter, cheese, and other dairy products can be produced from it are discussed. Comparative practices in various European countries and in the United States were outlined. A.H.J.

200. Shipping Milk in Track-Rail Steel-Lined Tanks.

ANONYMOUS. Ice and Refrig. *94*: 1, 71, 1938.

Some railroads, *e.g.*, the N. Y., Ontario and Western have transported milk in special 2,500 gallon steel lined tanks which are transferred from truck to flat cars. Four or five tanks are loaded on a single flat car and are again transferred to trucks on arrival in the city. The tanks are readily transferred and may be used as storage tanks in the plant. The inner stainless steel shell is covered with 2½ inches cork insulation and a steel outer shell. L.C.T.

201. Practical Value of the Resazurin Test. ANDREW MOLDAVEN. Milk Plant Monthly, *27*: 10, 58, 1938.

Tests were carried out with the purpose of finding out if the resazurin test was consistent from a practical standpoint, to grade milk supplies of "unknown" origin according to their sanitary and keeping qualities.

The results showed that advanced cases of mastitis, "wet" milking, milk handled in poorly sterilized utensils or cans, faulty cooling and other unsanitary conditions can be detected by the resazurin test.

The resazurin test also can be used to differentiate certain off-flavors in milk such as feed, from those due to bacterial action, by showing good keeping qualities of the former.

The simplicity, rapidity, and dependability of the test make it a valuable one in the improvement in the quality of raw milk supplies. G.M.T.

202. The Chloride Content of Milk. W. L. DAVIES, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res., *9*: 327–335. 1938.

Chloride analyses were made on 7802 samples of milk principally from the Shorthorn and Guernsey cows in the Institute herd, though results from a herd of Friesian and a herd of Ayrshire as well as other samples are included. The modal value for Guernsey and Shorthorn milk was found to be 71 to 80 mg. per cent and for the Friesian milk 111 to 120 mg. per cent. Morning milk was found to have a higher chloride content than evening milk as the mode for morning milk was 81 to 90 and for evening milk 71 to 80 mg. per cent. The data indicate that milk from successive lactations of a cow increases in chloride content. Periods of drought in summer caused an appreciable increase in the chloride content of the milk. The effect of changing from winter feeding to spring grass, however, was to lower the chloride content. With advancing lactation the chloride content increased slowly during the period when milk yield is relatively high. Usually after the seventh month, however, the chloride content of the milk rose rapidly.

The electrical conductivity at 18° C. of over 100 samples of milk of chloride content ranging from 61 to 235 mg. per cent was determined by the bridge method. The electrical conductivity of milk was found to increase with chloride content, however; in samples high in chloride the electrical conductivity increased at a greater rate than the chloride content. S.T.C.

203. The Methylene Blue Reduction Test and the Keeping Quality of Milk. C. H. CHALMERS, The University, Leeds, England. J. Dairy Res., *9*: 351–355. 1938.

The keeping quality of 200 samples of milk was determined as the time required for the milk to develop an unpleasant flavor on incubation at 15° C. (59° F.). The Methylene Blue Reduction test was made on different portions of the same milk samples after incubation for one hour at room temperature and after incubation for 7, 16, and 23 hours at 15° C. (59° F.).

The correlation coefficients for the reduction time and the keeping quality for the different age periods at which the reduction test was started were as follows:

Age of the milk when tested hr.	Correlation between the reduction time and the keeping quality
23	0.954 ± 0.0043
*16	0.893 ± 0.015
7	0.660 ± 0.055
1	0.442 ± 0.059

* Only 75 samples tested.

Thus the author concludes that by incubating the milk for a period of 15 to 24 hours at 15° C. (59° F.) a much better correlation can be obtained between the reduction time (at 37° C. (98.6° F.)) and the keeping quality, than if the milk is examined within a few hours of its production. S.T.C.

204. Physical State of Fat and Losses in Centrifuging. HAROLD E. ROSS, Milk Plant Monthly *27*: 12, 30, 1938.

Eighty-pound samples of milk were divided into two lots of 40 pounds each. One lot was cooled to 50° F. and held for 30 minutes, while the other lot was heated to 115° F. for 30 minutes. Both lots of milk were then adjusted to a temperature of 85° F. and run through the same separator.

Samples of cream and skimmilk were collected from each lot and tested for fat.

Thirty of 31 samples showed a greater loss of fat in the skimmilk from the samples warmed from 50° F. to 85° F. than the skimmilk from the samples cooled from 115° F. to 85° F.

The average difference of fat loss between the two lots of skimmilk was 0.028 per cent by the Mojonnier method. So far as could be determined the controlling factor was the physical condition of the fat. G.M.T.

MISCELLANEOUS

205. Carbon Dioxide in Its New Field of Usefulness. J. C. GOOSMANN, Dr. Eng., Carbonic Engineering, New York, N. Y. Ice and

Refrig. *91*: 6, 462; *92*: 2, 130; *92*: 3, 208; *92*: 4, 285; *92*: 6, 434; *93*: 4, 279; *93*: 5, 359.

This is a continuation of abstract previously reported. (See abstract Number 154, J. OF DAIRY SC., Vol. 20, No. 5.) Methods for the purification and dehydration of carbon dioxide gas, which represent two important steps in the manufacture of dry ice, are discussed in considerable detail. Equipment for solidification of liquid carbon dioxide is diagrammed and illustrated. A description, together with illustrations of the Mathieson Alkali Manufacturing plants, is included. L.C.T.

206. Survey of Cold Storage Locker Plants. ANONYMOUS. Ice and Refrig. *93*: 4, 271, 1937.

Operating data for 48 locker plants is included. The number of lockers vary from 100 to 625. Monthly rentals vary from seventy-five cents to $1.50, and yearly rentals from $7.50 to $10.00. Practically every plant has a butcher in charge. Locker plants are operated largely in connection with other enterprises, *e.g.*, creamery, ice and cold storage, service stations, etc. The population of towns where located varies from 200 to 52,000. Distances served range up to 20 miles. Lockers are rented mostly by farmers, although up to 40% of the space in some instances is rented to those living in the city. A few who report the cost of electricity and water estimate it at $1.50 to $2.20 per locker per year. A list of precautions to be observed in the successful operation of a locker system include such items as a butcher in attendance, sharp freezing of meat before it is placed in the locker, no seasoning be used in the meat, rapid handling, build a well insulated plant, not too large, avoid monthly renters, encourage a three month turnover of meat in order to avoid refrigerator burn, and investigate thoroughly before embarking on the enterprise. L.C.T.

207. Cold Storage Locker Plants. A. L. BLATTI, Chief Eng. Railways Ice Co., Chicago, Ill. Ice and Refrig. *92*: 5, 371, 1937.

The cold storage locker industry had its origin on the Pacific coast about six years ago. Now there are more than 6,000 in existence. The layout of a locker plant should consist of a processing room to be held at 34° F., a sharp freezing room at – 10° F. and a locker room maintained at + 10° F. Floor plans for locker plants are included. L.C.T.

208. Automatic Controls—Results Obtained from Their Modern Application. W. E. ZIEBER, York Ice Machinery Corp., York, Pa. Ice and Refrig. *94*: 2, 114; *94*: 3, 207, 1938.

The author discusses briefly the differences between hand, semi-automatic, and automatic control of refrigeration systems, and points out (a) that direct acting controls are those in which a fluid is used in a thermal element, which through a piece of tubing causes a pressure change directly on the valve or motor to be moved, either by hydrostatic pressure or gas pressure; and (b) that an air operated system consists of small thermally operated valves which pass air through them from a central system. The air pressure in turn functions upon the various pieces of equipment; and (c) finally a control system in which the thermal element makes or breaks an electric circuit, which moves the mechanism to bring the various parts into play.

The discussion is illustrated with a number of charts and diagrams showing detailed locations of controls as they might be used in a dairy plant.

The writer points out the changes that have taken place in controls in recent years. L.C.T.

209. Plant Problems, Their Importance and Solution. FRED J. HOPKINS. Ice and Refrig., *92:* 3, 219, 1937.

A list of points which should be observed in order to reduce operating costs in the manufacture of ice are included as follows:

1. The existence of non-condensable gases in the ammonia system.
2. Insufficient charge of ammonia.
3. Ammonia leaks.
4. Improper degree of flooding of evaporator systems.
5. Low brine level in freezing tanks.
6. Low specific gravity of brine.
7. Improper condition of brine (either acid or too alkaline).
8. Improper submergence of cans in brine.
9. Improper water treatment (or no water treating system).
10. Lack of properly calibrated instruments (gauges and thermometers).
11. Uninstructed operating personnel.
12. Operation of obsolete equipment.
13. Unbalanced plant conditions.

L.C.T.

210. Fiber-Like Merino Wool Made from Casein. ANONYMOUS. Milk Plant Monthly, *27:* 9, 46, 1938.

A synthetic fiber having the appearance of wool can be manufactured from casein by a process similar to that used in making rayon from cellulose. This fiber has a chemical composition almost identical with wool; is faintly yellow in color and closely resembles the best grade of washed and carded merino wool. It has the characteristic kink of wool, but lacks the scaly nature of wool fiber. Soft and harsh fibers may be made by varying the manufacturing process. Both the fineness and length of the fiber is regulated in the process.

It is believed that it can be manufactured to be sold at a price on a par with rayon. The agents used for casein wool manufacture are cheaper and may be used longer than those used in rayon manufacture.

Casein is made from skimmilk. Whether or not the skimmilk will be more valuable for the feeding of calves and hogs, or manufactured into fiber remains to be determined. G.M.T.

211. Sterilization By Radiation. O. F. Garrett and R. B. Arnold. Milk Plant Monthly, *27*: 8, 39, 1938.

The possibilities of sterilizing with ultra violet light are pointed out in this article.

Data are presented which show that great decreases in bacterial counts are encountered on surfaces exposed to ultra violet light. The effectiveness of sterilization depends upon the types of bacteria present, time of exposure and distance from the source of the rays to the object being treated.

It seems possible that the method may be applied to equipment and operations in the dairy plant. G.M.T.

212. Applications of Nickel and Its Alloys. F. Renaud. Le Lait *18*: 179, 1004–08, 1938.

The fabrication of various types of dairy equipment from nickel, inconel, and particularly from stainless steel (18% chromium and 8% nickel) is discussed. A.H.J.

PHYSIOLOGY

213. Observation on Specific Nutritional Factors in Lactation. S. J. Folley, W. Ikin, S. K. Kon, and H. M. S. Wilson, Nat. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. J. Biochem., *32:* 1988, 1938.

The results of this investigation indicate that rats can lactate normally on simplified diets. No evidence was obtained of the existence of specific dietary factors stimulating lactation qualitatively different from the dietary principles normally received by the non-lactating rat.

A simplified diet of ground rice, casein, butter, salts and yeast was found to produce satisfactory lactation in rats. Additions of fresh liver, liver extract or yeast were without effect on lactational performance. Fairly satisfactory lactational performance was obtained on a similar diet in which casein was replaced by fish protein. Stock colony rats which failed to rear their first litters were found to be more likely to lose their second litters than rats which successfully raised their first litters. Subcutaneous injection of tablets of pig anterior pituitary into such rats just after the second parturition had no effect on their ability to rear their young. K.G.W

214. The Diffusion of Trimethylamine Oxide from the Udder. W. L. Davies, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res., *9*: 323–326. 1938.

A neutral solution of pure trimethylamine oxide was injected into two quarters of an udder. The quarters increased in turgidity up to 2.5 hours and then relaxed. The oxide diffused rapidly out of the quarters during the third and fourth hours after injection, and a maximum concentration was shown in the urine at the end of the third hour. In a period of 8.3 hours, 76 per cent of the oxide available for diffusion was recovered from the urine. S.T.C.

215. The Composition of the Blood and Milk of Lactating Cows during Inanition, with a Note on an Unidentified Constituent Present in Certain Samples of Abnormal Milk. J. A. B. Smith, G. R. Howat and S. C. Ray, The Hannah Dairy Research Institute, Kirkhill, Ayr. J. Dairy Res. *9*: 310–322. 1938.

The yield and composition of the milk secreted by three non-pregnant Ayrshire cows during a 12 day fast were determined. During the fast the yield fell to about one-seventh of the original figure. The protein and fat contents of the milk of all three cows rose considerably, though variably. There were marked changes in the chloride-lactose ratio in the milk from two cows with one of which inanition was complicated by milk fever and with the other by serious loss of condition. There was a marked increase in the chloride content and a decrease in the lactose content of the milk from these animals. The sum of the values obtained for protein, lactose and ash was lower than the value obtained for solids-not-fat by as much as 1.9. The authors postulate the presence of an unidentified constituent. S.T.C.

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- Prussian Dairy Research Institute, Kiel, Germany
- State Agricultural Colleges and Experiment Stations
- The Royal Technical College, Copenhagen, Denmark
- United States Department of Agriculture

ABSTRACTS OF LITERATURE

BREEDING

216. Idaho Bull Associations. D. L. FOURT AND IVAN H. LOUGHARY. Idaho Agr. Exp. Sta. Bull. 223, Nov., 1938.

The bulletin is a detailed report based upon data assembled on 48 bull associations organized in Idaho between 1919 and 1938. Based upon the experiences of the Idaho associations the benefits of bull associations such as; better bulls for less money, less maintenance cost, longer service per bull, standard breeding program, better quality of progeny, and stimulation of interest in dairying, are carefully discussed and evaluated.

A thorough presentation is made of the problems of organization and management including financing the association, loss of members, blocks, and bulls, quality of bulls, efficiency of bulls, feeding and management of bulls, records, maintaining interest, disease control and Constitution and By-Laws. D.R.T.

CHEESE

217. Pasteurization of Milk for Cheese-Making. J. G. DAVIS. Dairy Ind. *4:* 2, 43, 1939.

Although the trend toward pasteurization of milk for cheese making is quite recent the possibilities of best treatment have been considered for at least forty-five years.

Consideration is given to each of the apparent advantages and disadvantages of pasteurization as it is related to the cheese industry and stresses the advantages of bacterial destruction, uniformity of product and increased yield.

Little difficulty has been experienced in Cheddar cheese manufacture when pasteurization has been employed but some difficulty has been encountered by the Swiss cheese manufacturers in America. The European makers have been very successful. The most trouble is experienced by the manufacture of semi-soft and soft varieties. Many experts have stated that it is impossible to make really good cheese of this type with pasteurized milk. If true, this may be ascribed to the more important roles of the general flora and enzymes of normal unheated milk. D.V.J.

DISEASE

218. An Evaluation of Some of the Tests Used in Detecting Mastitis in Dairy Cattle. A. C. FAY, Director of Laboratories, H. P. Hood & Sons, Inc., Boston, Mass. J. of Milk Techn. *1:* 4, 38, 1938.

The paper discusses only the chronic form of mastitis. The paper presents an analysis of data from various sources giving a comparison of various "field" and "laboratory" tests for detecting mastitis.

The analysis shows that the percentage of agreement of the various tests with the isolation of streptococci to be in the following order.

1. Microscopic examination of a smear from incubated milk 87%.
2. The Hotis test 63%.
3. Enumeration of leucocytes 55%.
4. Manual palpation of the udder for detecting fibriotic tissue 40%.
5. The catalase test 39%.
6. The chloride test 38%.
7. The brom-thymol-blue test 36%.
8. The strip-cup test 26%.
9. Microscopic examination for streptococci in the unincubated milk 14%, and
10. Abnormal (purulent or watery) milk 8%.

These tests fall into three rather well defined classes:

1. The microscopic examination of the incubated milk which is fairly dependable when applied to known positive cases of mastitis and also to milk from cows known to be free from the disease.

2. The Hotis test, the enumeration of leucocytes, the examination of the udder for fibrosis, the catalase test, and the chloride test rarely give a false test on a positive sample, but milk from one third to two thirds of the cows known to be free from the disease will give a false positive test which does not necessarily indicate that the disease exists.

3. The brom-thymol-blue test, the strip-cup test, the microscopic examination of the direct smear for streptococci, and the observance of purulent, watery abnormal milk are characterized by failing to point out a sufficiently high percentage of the known positive cases to warrant their use as the sole means of identifying mastitis cows for segregation from the herd. L.H.B.

219. Die Feststellung der Abortus-Bang-Infektion (Bruzellose) des Rindes mit Hilfe serologischer Verfahren. II. Mitteilung: Die Schnellund Langsamagglutination mit Blut und Milch. (Serological Methods of Diagnosing Brucellosis in Cattle.) SEELEMANN UND WOLF. Zschr. Infektkr. Haustiere *54:* (½) 23–44.

Most of this report is on the evaluation of three methods of diagnosis—(1) the rapid agglutination test, (2) the slow agglutination test and, (3) the flocculation reaction (Meinickreaction). Although most of the tests were made on blood and blood serum, milk and milk serum were used in some instances. A rather long discussion of the literature is included.

The conclusions are briefly, that the rapid agglutination test with serum, when used in the laboratory, is a very sensitive and satisfactory method of diagnosis, but not so satisfactory outside the laboratory. The rapid test was usually read as positive in about one minute and as negative in five minutes. This test was found to agree fairly well with the flocculation test. The flocculation test is rather slow and may be improved by warming. When the tube test is taken as a standard the rapid test is somewhat more accurate than the flocculation reaction. There is a slightly closer correlation between the tests when milk constituents are used instead of blood.

A combination of tests appears to be of value in determining the diagnosis. When the rapid test was complete and the tube test exhibited a titre of 50 or more, the test was read as positive. When the rapid test was complete, the tube test was below 50 and the flocculation test positive, the reading was still considered positive. But when the rapid test was complete, the tube test below 50, and the flocculation negative, the case was reported as negative. L.D.B.

FEEDS AND FEEDING

220. Palatability of Soybean Meals for Dairy Cows. C. F. Monroe and C. C. Hayden. Ohio Agr. Exp. Sta., Bimonthly Bulletin XXIII, No. 195, pp. 209–214, Nov.–Dec., 1938.

Several palatability trials were conducted with soybean meals representative of those found on the market.

In one trial in which the meals were fed alone, free-choice, a decided preference was shown for expeller meal. Hydraulic meal was second and extracted meal third. Factors other than palatability may have influenced these results.

In one short trial (28–57 days) in which the meals were incorporated in grain mixtures to the extent of 20 per cent, no preference was shown. In a similar trial of longer duration (83–179 days) the order of preference was: extracted, expeller, hydraulic. In a fourth trial a heat-treated extracted meal replaced the hydraulic meal. The grain mixture containing the extracted meal was again preferred, the expeller mixture was second, and the heat-treated extracted meal was a poor third. However, when the grain mixture containing 20 per cent of the heat-treated extracted meal was fed to the same cows and no choice was allowed, it was consumed in normal quantities. W.E.K.

ICE CREAM

221. Report of Committee of Ice Cream Sanitation. International Assn. of Milk Sanitarians, F. W. Fabian, Chairman. J. of Milk Techn. *1:* 4, 20, 1938.

This report deals largely with fountain sanitation, counter freezers and chemical sterilization.

The subjects of total food solids standards for ice cream and controlling overrun are discussed.

Possible contamination due to materials added after pasteurization and methods for solving the problem are given.

The proposed new media and incubation temperature for standard agar plate counts are discussed. L.H.B.

222. Preparation of Ice Cream Mixes for Home Consumption. W. J. CORBETT AND P. H. TRACY, Dairy Husbandry Dept., Univ. Ill., Urbana, Ill. Food Research *3:* 6, 637, Nov.–Dec., 1938.

Studies dealing with the manufacture of a canned, sterile ice cream mix for home freezing indicated that such a product should be sterilized using a high temperature–short time procedure, should contain not more than 36 per cent total solids and 9 per cent serum solids, should be homogenized at medium pressures using two stages and should be flavored (if flavors are incorporated) with those which do not affect the heat coagulation point. F.J.D.

223. Chemical and Bacteriological Studies on Ice Cream. CECILY R. GRUMBINE AND EVELYN G. HALLIDAY, Home Economics Dept., Univ. Chicago, Chicago, Ill. Food Research *3:* 6, 653, Nov.–Dec., 1938.

A chemical, bacteriological, physical and economic analysis of different brands of vanilla ice cream sold in the Chicago area. F.J.D.

MILK

224. Sanitary Problems in Connection with the Use of the Homogenizer. W. J. CORBETT, Dept. of Dairy Husbandry, Univ. of Ill., Urbana, Ill. Milk Dealer *28:* 4, 42–46, 1939.

Bacterial counts on regular and homogenized milk at the University creamery are given.

Data are given showing that when pasteurized milk with an average bacterial count of 1182 was homogenized, the average count of the homogenized milk at the beginning of the run was 1980, in the middle of the run 1510, and at the end of the run 1420. When the average bacterial count of the pasteurized unhomogenized milk was 4125, the count for the homogenized milk was 12,125, 8775, and 6575, respectively, at the beginning, middle, and end of the run.

When sterile milk was homogenized there was a certain amount of "pick-up" by the homogenizer but not nearly as much as the increase in bacterial count in the case of the high-count milk which was homogenized. That the increase in bacterial count of milk, when homogenized, is due to the breaking up of bacterial clumps was shown by inoculating sterile milk with *staphylococcus aureus* and with *B. coli.* The milk inoculated with staphylococcus, which exists in clumps, practically doubled; while that inoculated with *B. coli*, which exists singly or in pairs, showed no significant increase in bacterial count when homogenized.

Data are presented showing that where milk was homogenized prior to pasteurization there was some increase in bacterial count and that the keeping quality of homogenized milk, as measured by increase in bacterial count, was not equal to unhomogenized milk. The latter is explained on the basis that during the homogenizing process some rapid-growing organisms are picked up and multiply very rapidly when the milk is held at temperatures above 50° F. C.J.B.

EDITOR'S NOTE:—In all cases the homogenizer used in this study was carefully washed, dried with compressed air and sterilized with chlorine before using so as to reduce bacterial contamination to a minimum. The new improved type of high pressure machine was used.

225. Observations on the Development of Rancidity in Sweet Milk, Cream, Butter. J. L. KEITH, Oklahoma A. & M. College, Stillwater, Okla. Milk Dealer *28:* 4, 82–84, 1939.

By comparing milk from cows known to produce milk which became rancid regularly with that from cows known to produce normal milk, the following facts were brought out:

When the rancid milk was separated, the off-flavor was intensified in the cream and in most instances, completely absent in the skim milk.

The separator was equally efficient in removing fat from normal and rancid milks.

The rancid samples of cream foamed more profusely than the normal samples and in most instances required a longer time to churn.

No significant differences were noted in the size, shape, or number of fat globules of the normal and rancid samples.

A greater degree of hydrolysis was definitely indicated in the rancid samples.

Churning cream from the cows which produced milk which became rancid was accomplished with less difficulty if the cream was pasteurized or churned raw before the off flavor developed.

The butter became rancid and otherwise off-flavored immediately or within a few days in storage at 40° F.

The flavor of the milk of the college herd was noticeably improved when milk which developed rancidity was not included. C.J.B.

226. Better Milk Production. Anonymous. Milk Dealer *28:* 4, 48–52, 1939.

A description of the part which 4-H dairy clubs are playing in teaching better methods of milk production. The outline of a typical demonstration is also given. C.J.B.

227. **Controlling Cream Quality in the Plant.** N. E. LAZARUS, President, Lacteal Analytical Laboratories, Buffalo, N. Y., Milk Dealer *28:* 5, 34–35, 68–72, 1939.

The causes and methods of preventing plug formation, oiling off, serum separation, poor viscosity, high bacterial counts, off flavors, poor whipping, lipolytic enzyme activation, feathering, air incorporation, high acidity, and destruction of fat clumps in cream are discussed. The following rules are given for the production of fine quality cream.

1. Use only a good quality raw milk supply.
2. Avoid agitation and air incorporation from raw supply to finished product.
3. Do not use obsolete apparatus designed for handling milk or by-products.
4. Use pasteurizers to at least 70 per cent capacity.
5. Do not use over capacity pumps with high r.p.m. but synchronize the flow of milk and cream with positive type pump.
6. Cool cream only to 48 degrees F. and allow to cool down to 38 degrees F. in a 24-hour aging period in the cooler, without agitation.
7. Handle cream gently at all times.
8. Separate cream at 80–84 degrees F. for increased viscosity.
9. Use recording thermometers—don't guess at temperatures. C.J.B.

228. **Research with Cream at the Massachusetts State College.** MERRILL J. MACK. J. of Milk Techn. *1:* 4, 50, 1938.

This paper enumerates the desirable characteristics of cream. Factors causing oiling off of cream in coffee and means of preventing are discussed as are the whipping properties of cream.

In conclusion, it is stated that, in general, defects in cream are due to excessive agitation in processing, too rapid heating, or reprocessing. Cream should be handled the minimum number of times, carefully pasteurized, and cooled, and marketed when fresh. L.H.B.

229. **What the Colleges Are Doing in the Training of Milk Sanitarians.** FORDYCE ELY, Prof. of Dairy Husbandry, Univ. of Ky., Lexington, Ky. J. of Milk Techn. *1:* 4, 43, 1938.

This paper gives data obtained mostly from a questionnaire sent to the heads of dairy departments in the various land-grant agricultural colleges in the U. S. Response came from 37 states.

The data showed that an average of 18 credits is offered in specialized dairy courses in the various colleges of agriculture which are considered to be extremely valuable in training milk sanitarians. About 175 students are graduated each year who have taken these courses, but during the past five years only about one-sixth of them have entered this field of work.

Thirteen states offer short courses or conferences valuable for training milk sanitarians. L.H.B.

230. Modern Methods of Milk Plant Inspection. MAURICE H. SHORAGO, Consultant to the Dairy Industry, Boston, Mass. J. of Milk Techn. *1:* 4, 32, 1938.

A discussion of points needing especial attention, and tests which will aid an inspector in doing a more thorough job of inspecting are given. L.H.B.

231. Practical Farm Sterilization. ARTHUR H. WILLIAMSON, State Dairy Supervisor, Florida State Board of Health, Jacksonville, Fla. J. of Milk Techn. *1:* 4, 14, 1938.

This paper gives a thorough discussion pertaining to the construction of steam chests for use on dairy farms. L.H.B.

232. The Problem of Recontamination of Pasteurized Milk and Its Products. L. C. BULMER, Director, Bureau of Food and Dairy Inspection, Jefferson County Board of Health, Birmingham, Ala. J. of Milk Techn. *1:* 4, 10, 1938.

The fact is stressed that much effort has been expended in obtaining proper pasteurization of milk and dairy products, but little effort has been made towards preventing recontamination of the products.

It is advocated that the product should flow directly from pasteurizer to the final container. This would include ice cream mix and would prohibit the freezing of ice cream mix in any establishment other than where it was pasteurized.

Covered lips for milk bottles and dating cap are also advocated.

The U. S. Standard Milk Ordinance is also discussed. L.H.B.

233. A Colorimeter for the Phosphatase Test. W. H. BOYNTON AND P. E. NELBACH, Dept. of Public Health, Yale Univ., School of Medicine, New Haven, Conn. J. of Milk Techn. *1:* 4, 8, 1938.

Directions and diagrams are given for making a colorimeter for use with the Gilcrease and Davis standards for the phosphatase test. L.H.B.

234. The Cleaning of Milk Utensils. L. H. BURGWALD. Ohio Agr. Exp. Sta., Bimonthly Bull. XXIV, No. 196, p. 4, Jan.–Feb., 1939.

Bacterial counts made on 5-gallon milk cans, some of which had been kept dry after washing and others wet with sterile water, showed that in 24 hours at room temperature the wet cans contained many more bacteria than just after washing, whereas the cans that were kept dry showed prac-

tically no increase in bacteria. It was estimated that milk put into the wet cans after 24 hours would be contaminated with 6,680 bacteria per cc.
W.E.K.

MISCELLANEOUS

235. Dairy Detergents: Their Analysis and Valuation. W. L. Davies. Dairy Ind. *4:* 1, 3, 1939.

Simple tests for determining detergent strengths of soaking-tank solutions and simple tables for solution standardization are recommended to increase the efficiency of bottle cleaning and sterilization.

In evaluating the detergent strength of a solution the "effective alkalinity" is taken as a rough index. All of the compounds of an alkali mixture are evaluated on their equivalent as caustic soda (caustic soda effective alkalinity considered 100). A pH of 11 is considered the minimum alkalinity tolerated by bacteria. Because of the dilution of soaking-tank solutions a solution with a pH well above the minimum is desirable. The effective alkalinity of the common basic washing compounds (referred to 98 per cent caustic soda with an effective alkalinity of 98 per cent) are, 58 per cent soda ash—29 per cent alk., sodium meta silicate—44 per cent alk. and tri sodium phosphate—18 per cent. Sodium meta silicate and tri sodium phosphate show up unfavorably because of their high percentage of water of crystallization.

Analytical methods are described and the analyses of a number of commercial detergent mixtures are shown. D.V.J.

236. Chemical Conservation of Manure. C. J. Schollenberger. Ohio Agr. Exp. Sta., Bimonthly Bull. XXIII, No. 195, pp. 219–220, Nov.–Dec., 1938.

In a discussion of the advisability of using phosphoric acid or superphosphate for preventing ammonia losses from manure, it is shown that such a procedure is unprofitable. The use of acid peat moss appears to be a more promising means for conserving nitrogen in manure than does the addition of chemicals. W.E.K.

PHYSIOLOGY

237. Thyroid Stimulation by Cold Including the Effect of Changes in Body Temperature upon Basal Metabolism. G. C. Ring, Dept. of Physiology in the Harvard Medical School, Cambridge, Mass. Am. J. Physiol. *125*: 2, 244, 1939.

Living for three weeks or more at 0° to 5° C. produced in rats an average elevation in metabolism of 21 per cent. Corrected for changes in body temperature this figure becomes 16 per cent. This increased metabolism is brought about largely if not entirely by the thyroid gland. Nervous pathways through the superior cervical ganglia are not essential for bringing about stimulation of the thyroid gland by cold. D.L.E.

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Proceedings of Society of Experimental Biology and Medicine

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Tierernährung
Tierzüchter
Trudy Vologodskogo Molochnogo Institut

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Züchtungskunde

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BACTERIOLOGY

238. The Implantation of Oral and Intestinal Strains of L. Acidophilus in the Albino Rat. MICHEAL J. PELCZAR AND LUTHER A. BLACK, J. Bact. *37:* 1, 51–67, 1939.

Seven oral or dental strains of *L. acidophilus* were studied and none were found to be implantable in the intestine of white rats. Four of the five rough intestinal strains were found to be implantable. Rough intestinal strains were found to be more readily implanted and persisted in the intestine for a longer period of time than did the smooth intestinal strains. *L. bulgaricus* was found not implantable which is in accord with previous studies. E.H.P.

BUTTER

239. The Cooking-residue of Butter. W. RITTER, Schweiz. Milchzeitung No. 47, 1938.

The cooking residue of butter represents the condensed butter serum, which has been, so to speak, fried in the butterfat. It has a pleasant smell and taste and people like to eat it for instance spread on cake, or as an addition to fried potatoes. This cooking residue is composed of the lactose, the albumen and the salts of the butter. The relatively high content in butter phosphatides is particularly characteristic; the cooking residue of butter represents certainly the dairy product most rich in phosphatides that we know. It contains further 30–60 per cent of butterfat and varying amounts of water. On cooking sweet butter the cooking residue is quite sweet; acidified butter gives a residue with a pleasant sourish, and salted butter a residue with a salty taste. Lactose is present probably in the form of lactose anhydride. W.R.

240. Microscopical Examination of the Crystallization of Cooked Butter. W. RITTER, Schweiz. Milchzeitung No. 76, 1938.

Microscopical and polarisation microscopical examination of butterfat proved the following:

When it is cooled slowly, without stirring, or rapidly, in thick layers, butterfat forms centrally united bunches of needles, in which hundreds and thousands of crystal needles radiate from one point in all directions. These big, hedgehog like crystals are enclosed in an easily melting part of the butterfat, the butter-oil. They therefore do not form a solid mass, but are suspended more or less in the liquid basic substance. On the other hand finely crystallized butterfat, as it is obtained by the inoculation method, consists in small single crystal needles which are entangled in every direction. Therefore the solid phase of the butterfat forms the basic substances in this case, while the liquid part, the butter-oil, is distributed between the little crystal needles. Hedgehog like crystals are always a symptom of bad crystallization, whereby insufficient solidity is prevailing, owing to the liquid basic substance. A slight warming (summer temperature) is enough to increase the amount of butter-oil, so that the coarse crystals fall to the bottom. If, on the contrary, the butterfat is cooled, the butter-oil concentrates; the upper parts of the hedgehog like crystals, spreading apart, consolidate and air is allowed to penetrate; thereby a white color is produced which is objected to by the consumer. On warming the fat the butter oil expands again, the air is removed and the normal color of the fat reappears. When the fat is heated more, the oil appears on its surface in the form of "tears." Such coarse, hedgehog like crystals have practically no inoculative effect if added to liquid fat cooled at 30–32° C. They may, however, be transformed into inoculative material by being carefully warmed at 36° C. under continuous stirring; thereby the accumulations of crystals separate in single crystal needles which may then initiate a good crystallization. W.R.

241. The Foaming of Fats, Especially Butter, When Used for Baking.

W. Ritter. Mitteilungen Lebensmittel-Untersuchung & Hygiene *29*, 253–261, 1938.

Occasionally samples of butterfat are found which are foaming exceedingly when used for instance for the preparation of potato-chips. This foaming is due to an increased content in butter phosphatides. When butter or other phosphatides are added to oils or fats which do not foam, this phenomenon can be produced by heating the mixture with wet potatoes.

When cooked butter (ghee) is prepared, small amounts of butter phosphatides will pass, towards the end of the cooking process, from the cooking residue into the hot fat. They generally are separated again in a flaky or slimy form, when the butterfat is cooled at about 42° C. or when it is allowed to congeal and carefully warmed afterwards at 42° C. Then the phosphatides can be removed by filtration through paper. When the fat is heated anew, the phosphatides are dissolved again and run clear through the filter with the fat. There are sometimes samples of butter of a rather brown color, rich in phosphatides which they do not eliminate when cooled, etc. In such cases the phosphatides can be partially removed by a treatment with bone-black or other means by adsorption; this implies, however, that the butter pigment (carotene) is removed too. Better results are obtained by adding small amounts of water to the butterfat (less than 1 per cent). If well distributed in the butterfat, the water becomes absorbed by the butter phosphatides which are thus "hydratised" and made insoluble in the butterfat. The phosphatides are removed by filtration in a swelled up state and the originally brown fat assumes a normal yellow color; at the same time there will be no more foaming, when the fat is heated with wet potatoes. The precipitation of the phosphatides which occurs quite normally when cooked butterfat is cooled, is due to its water content. The water being dissolved in the hot fat, a certain amount of phosphatides may be dissolved too. On cooling the hot fat, part of the dissolved water becomes insoluble, separates out and "hydratises" the dissolved phosphatides; these become insoluble too and are precipitated in a flaky or slimy form, according to their quantity and the water content of the butterfat. When butterfat is obtained by melting butter at about 42° C., it will be free from phosphatides, because the latter, if present in the fat, would have been immdiately "hydratised" by the water of the butter serum and made insoluble in the fat.

W.R.

242. Oxidation of Butterfat. I. The Formation of Peroxides in Butterfat. W. Ritter and Ths. Nussbaumer. Schweiz. Milchzeitung No. 12, 1938.

The beginning of the oxidation of butterfat can quite easily be fixed by determining the peroxide number. This latter, according to Lea, increases to a great degree when the fats are exposed to light. When air is made to pass through heated butterfat, the peroxide number increases more rapidly than without air. Copper, used as fat soluble copper palmitate, accelerates the assimilation of oxygen and increases accordingly the peroxide number of the fat, when this is stored in the dark at normal temperature. The higher the storing temperature, the quicker the increase of the peroxide number. The constancy of butterfat against oxidation is examined by heating 10 grams of the fat at 104° C. in a Petri dish during 8 hours and determining afterwards the peroxide number. Great differences will thereby result when butterfats of different constancy are examined. In general a butterfat is all the more constant against oxidation, the less the peroxide number increases in this test. Oxidizing fats distinctly grow in weight when heated. Therefore the determination of the percentage of water in butterfat must be carried out in the vacuum-drying stove. Fat extracted according to the Röse-Gottlieb method very often shows an extremely rapid increase of the peroxide number. If hydroquinon is added, the assimilation of oxygen in the light or while heating the butterfat is deferred for sometime. Butterfat obtained by melting butter at a low temperature gives a greater and quicker increase of the peroxide number than properly cooked butter. When tallowy oils or fats are intensely heated (for instance at 250° C.), the peroxide number decreases, probably because the peroxides of the fat are destroyed, that is to say, reacting with other groups of the fat. If, on the other hand, normal butterfat is heated for a short time at 250° C., it will show afterwards at normal temperature or at 42° C. a quicker increase of the peroxide number than the same but not over heated fat. We may therefore conclude that overheating destroys certain elements contained in butterfat which are important for its keeping quality. W.R.

243. Oxidation of Butterfat. II. The Influence of Copper on the Peroxide number of the Butterfat. W. RITTER AND THS. NUSSBAUMER. Schweiz. Milchzeitung, No. 38, 1938.

It is demonstrated that an addition of increasing quantities of fat soluble copper palmitate to butterfat causes the peroxide number to increase all the more rapidly during the storage of the fat in the dark at ordinary room temperature, as the copper concentration is higher within the chosen range of 0.000001 to .33 per cent. If, on the other hand, the copper containing fat is stored at ordinary room temperature in the light, it shows a slower increase of the peroxide number than fat which is free of copper. Later on the peroxide number of copper containing butterfat decreases in spite of a continued exposure to light, which is not the case with copper free fat.

On heating the samples during 8 hours at 104° C., fats which contain about 0.0001 per cent of copper palmitate reveal the greatest and fat with a greater copper content a less important increase of the peroxide number. The reason for this phenomenon is that copper does not only accelerate the assimilation of oxygen on increasing the peroxide number, but also the further conversion of the fat peroxides. That is why the peroxide number decreases again in the presence of greater amounts of copper as well as in the light and in the warmth. Moreover, a distinctly tallowy butterfat, when heated at 104° C. during 8 hours, decreases all the more its high peroxide number, the higher the addition of copper palmitate. The nature of the second copper catalysis bringing about the destruction of the fat peroxides is not fully known. W.R.

244. Oxidation of Butterfat. III. The Influence of Different Substances on Peroxide-formation in the Butterfat. W. RITTER AND THS. NUSSBAUMER. Schweiz. Milchzeitung No. 82, 1938.

The influence of the peroxide number of different kinds of butterfat and the influence of additions to the butterfat were examined by heating 10 grams of the fat during 8 hours at 104° C. in a Petri dish of 10 cm. diameter. It resulted that butter shows a much slower increase of the peroxide number than pure butterfat. Whole butter very often has not oxidized after 24 hours to the same degree as pure fat after 8 hours. While the peroxide number of the pure butterfat rises herewith at about 40 on an average, that of the whole butter is only 1–3. This result is not due to evaporation of water, for it is scarcely affected by adding to pure butterfat an equal quantity of water as contained in the whole butter. The butter serum must therefore contain elements which slacken the oxidation of butterfat. Properly cooked butter shows a considerably slower increase of the peroxide number than butterfat obtained by melting the butter at low temperatures. An ordinary cooking residue reduces the oxidation of the butterfat. A cooking residue extracted by benzol (removal of fat and butter-phosphatides) slackens but feebly the increase of the peroxide number. Lecithin and particularly cephalin obtained from plant lecithin or butter lecithin manifest a strong antioxidizing effect; dried egg yolk which is rich in lecithin does the same. "Avenex" added to butterfat in a dose of 1 per cent also produces an antioxidizing effect.

The antioxidizing effect of buttermilk and buttermilk-powder is relatively slight; on the other hand that of butter serum obtained by melting of butter is very strong. It follows that, on melting the butter, its antioxidizing elements are not transferred to the butterfat, but to the serum. Butter serum has a considerably higher phosphatide content than buttermilk and this is probably one of the reasons for the antioxidizing effect of butter serum. It follows equally from these statements that butter is more constant against oxidation than butterfat. Conditions in acidified butter are not yet cleared. W.R.

245. Oxidation of Butterfat. IV. Influence of Fat-peroxides on Keeping Quality of Butterfat. W. Ritter and Ths. Nussbaumer. Schweiz. Milchzeitung No. 92, 1938.

Oxidation may progress gradually from tallowy parts in the butterfat. This is due to the autocatalytic course of fat oxidation, which is accelerated by the developing fat peroxide. According to that the peroxide number increases slowly at first and more and more rapidly afterwards, until it has to slacken increase again. For this reason small amounts of already oxidized fat will reduce the keeping quality of normal butterfat. This was demonstrated by pursuing the course of the increase of the peroxide number in butterfat on adding 0, 1, 2, 5, and 10 per cent of tallowy butterfat at 20, 37, and 50° C. The influence of the oxidation accelerating effect of the fat peroxides became distinctly manifest, but it was not stronger than that of the storing temperature. The time needed for the peroxide number to reach a value of about 5–10 is very important for the determination of the keeping quality of butterfat. Later on, the natural protecting elements of the butterfat are no longer decisive for the assimilation of oxygen, the reaction taking an autocatalytic course. As it is difficult to fix the moment when the peroxide number reaches 5, the increase within a given time must be taken as a basis or else the time must be determined by means of the increase curve of the peroxide number. The turning white of the butterfat may serve as a hint that the peroxide number has approximately reached 5–10.

W.R.

246. The Congelation of Cooked Butter (Ghee). W. Ritter. Schweiz. Milchzeitung No. 93, 94, and 96, 1937.

In order to be solid and not to separate when exposed to warm summer temperatures butterfat must be as fine grained as possible. This is realized by cooling the hot butterfat at 30–32° C. and inoculating it with fine grained solid butterfat. After some time the mass, which is assiduously stirred, becomes thick and can be filled into the tins. If the butterfat is not properly cooled, coarse grains are formed which are suspended in a basic mass of liquid butter oil. It consequently occurs especially during warm summer temperatures that butter oil flows out of the tins and that a sabulous sediment is formed; this latter process is still hastened by a quick cooling at low temperature. When such a fat is warmed up, the butter oil sometimes appears at the surface of the mass in form of "tears." Butterfat that has not been duly cooled is liable to remain liquid at temperatures below 20° C., that is to say it can be poured from one tin into another and stirred as one likes. Properly cooled butterfat, on the contrary, is quite solid at those temperatures. The cooling of the fat, filling into tins and preparation of the inoculative material, etc., are described in detail. W.R.

247. About the Ways of Acting of Butter Made of Milk and of Butter Made of Separated Whey in Enzymic Reactions (Aldehyde-reductase- (Xanthine-oxidase-) reaction and Peroxidase-reaction). W. Ritter. Mitteilungen Lebensmitteluntersuchung und Hygiene *28*, 197–205, 1937.

Literature gives the following characteristics for butter made of milk and for butter made of separated whey in enzymic reactions: Milk-cream-butter (non-pasteurized) reduces immediately a mixture of formaldehyde and methylene blue, while butter made of separated whey and of whey the fat of which is collected by acidifying and heating (Vorbruchbutter) reduces very slowly or not at all. Milk-cream-butter and butter made of separated whey give a positive Storch reaction which takes place also, though more slowly, in butter made of whey the fat of which is collected by acidifying and heating. That butter made of separated whey which is not heated reduces but slowly a mixture of formaldehyde and methylene blue is therefore unexpected. On the other hand butter made of whey, the fat of which is collected by acidifying and heating, produces a positive Storch-reaction after the latter procedure.

According to the experiences of the author the copper-content of both kinds of butter is responsible for these anomalies, since they are in close contact with the copper kettle during the manufacturing process.

On examining the whey cream of cheese prepared in exactly the same whey, but in a copper kettle in one case and in a kettle of stainless steel in the other, it was stated that the above mentioned anomalies did not occur in the latter case. The whey cream proceeding from the kettle of stainless steel showed a normal reduction time and no positive Storch reaction took place after the heating at curdling temperature. This cream did not develop tallowy flavor during cold storage, as was the case with the cream proceeding from the copper kettle. The aforesaid enzymic reactions applied for the differentiation of the kinds of butter are therefore only available for butter made in copper kettles, not for butter made in kettles of aluminium, tinned material or stainless steel. W.R.

248. The Importance of Pasteurization Temperature for Butter Making. W. Ritter. Alpenländische Molkerei- und Käserei-Zeitung *11:* 159–161, 1937.

On judging the effect of pasteurization on the quality of the butter, it is not only the bacterial result that must be taken into account. Other sides of the problem, the more chemical ones, are equally if not more important. So it was revealed by extensive experiments that butter made of cream pasteurized at 90° C., did not develop fishy flavor when stored, although the cream had been thoroughly acidified; if, however, the cream was heated at 78 to 82° C. only, the butter became fishy during the storing under otherwise equal conditions. This phenomenon cannot be caused by the destruction of the lecithin, the cooking residue of such a butter showing still a relatively high lecithin-content even after a long cooking at high temperature. It is also mentioned that analogous conditions arise on preparing other dairy products. W.R.

249. The Cooking of Butter. (Ghee-manufacturing.) W. Ritter. Reports of the World's Dairy Congress, Berlin, *2:* 156–161, 1937.

A short report is made on the ghee-manufacturing, the foundations and ways of cooking the butter, the phenomena occurring during the cooking-process, the keeping quality of butterfat and its ways of acting during the heating. Further on the differences between butterfat obtained by different methods, the crystallization of butterfat, the water content of cooked butter (ghee), the phosphatide content of differently obtained butterfats, bacterial defects, chemical defects of the product offered for sale, experiences made on controlling cooked butter (ghee). W.R.

250. The Testing of Starters in Dairy Plants. W. Ritter. Reports of the World's Dairy Congress, Berlin, Vol. *2,* 104–07, 1937.

Besides the examination of appearance, flavor and smell, the following methods of testing may be easily applied by dairy plants: Test on bacterial pureness according to Burri's smear culture method; test on development of carbonic acid by shaking the culture in a flask; determination of the degree of acidity, of the volatile acids and also of diacetyl by the Voges-

Proskauer method in the distillate of the determination of volatile acids, after their neutralization. The determination of diacetyl is carried out by steam distillation of 50 ccm. of the starter, 50 cc. of water and 3 cc. of N-sulphuric acid. Two samples of 10 cc. each of the distillate are titrated with n/20 solution of sodium hydroxide and phenolphthalein and tested on diacetyl by an addition of creatine and sodium hydroxide solution, eventually also α-naphthol solution.

A consumption of 0.60–0.85 cc. of N/20 sodium hydroxide solution obtained by neutralization of 10 ccm. of the distillate reveals a good starter, while lower figures indicate that the aroma streptococci have been restrained. When larger amounts of volatile acids are produced, acetone and diacetyl undergo in most cases further transformations, so that the reaction becomes more feeble or negative. W.R.

251. The Means of Preventing Fishy Flavor. W. Ritter. Reports of the World's Dairy Congress, Berlin, Vol. 2, 162–167, 1937.

Fishy flavor occurs only in stored butter made of acidified cream. Therefore, acidification of the cream is generally omitted or the latter is neutralized before the churning, in order to prevent fishy flavor. The use of antioxidants, for instance hydroquinon, too would check this defect, but this is not legal. Certain bacteria are able to prevent oxidative changes in milk, cream, and butter. The most important method is, however, the application of an increased pasteurization temperature. Practical experiments carried out on a large base (6000 litres a day) in the central butter plant at Lucerne have revealed that fishy flavor developed when the cream was heated at 78, 80, or 82° C. in the flash pasteuriser; it did but rarely occur when the cream was heated at 86° C. and practically never appeared when the cream was pasteurized at 90° C. Similar observations were made in other butter plants on occasion of the taxations. These results are due to a chemical, not a bacterial effect of pasteurisation temperature. W.R.

252. Holding Cream Raw and Pasteurized for Forty Hours. C. C. Totman, Am. Prod. Rev. 742–44.

Churnings of cream were neutralized and pasteurized and held 15 hours before churning; a second series were neutralized, pasteurized, and held 40–45 hours; while a third was held raw 40–45 hours, then neutralized, pasteurized, held two hours and churned. Cream held raw yielded higher scoring butter both when fresh and after six months' storage. This was especially true in the cases of lower grades and high acidity creams. With good cream the method of handling had little, if any effect. Several reasons for the results may be advanced: antagonistic action of lactic acid bacteria to proteolytic and lipolytic bacteria, and chemical action in the cream serum due to the effects of neutralizer, metallic salts, and heat. P.S.L.

253. How Fat Losses May Be Reduced. S. T. Coulter, Am. Prod. Rev. *85:* 25, 740–41, 1938.

Data are given from the operation of the Minnesota State Experimental Creamery. The author stresses three fundamentals: analysis of buttermilk from each churning and calculation of per cent butterfat lost of total fat received; training of employees in record accuracy and methods for reduction of fat losses; and, preparation of a chart to show weekly fat losses.
P.S.L.

254. A Skunk-Like Odor of Bacterial Origin in Butter. T. J. Claydon and B. W. Hammer, J. Bact. *37:* 3, 251–258, 1939.

From unsalted butter, which on holding at 21° C. developed a skunk-like odor, an organism, *Pseudomonas mephitica,* was isolated. This organism when inoculated in cream, pH 5.0–7.5, and churned, developed the defect in unsalted butter. Salt was found to have an inhibitory effect. The flavor could also be produced in unsalted butter by inoculating small quantities of a culture of *Ps. mephitica* into water used to wash butter. E.H.P.

255. An Anti-Oxidant for Dairy Products. C. D. Dahle, Pennsylvania State College, State College, Pa., Nat. Butter and Cheese J., *29:* 24, 8, 1938.

Oxidized flavor is the most serious of all flavor defects of food. This flavor defect arises from the oxidation of fat. Oxidized flavor is associated with copper contamination of dairy products; exposure of such products to sunlight; the activity of an enzyme; and with the production of milk of low bacterial content. Cream, frozen for ice cream, is not susceptible to this defect. After all other precautions have been taken to prevent the occurrence of this flavor it may be still further inhibited by adding to the cream approximately 2 per cent of a specially prepared oat flour. Parchment paper, specially treated with oat flour, is recommended for preventing surface staleness in butter. Addition of oat flour directly to cream for buttermaking tends to prevent oxidized flavor. A water extract of the flour is recommended to avoid sediment in the butter. Oat flour also delays the formation of other flavor defects in butter, such as stale and cheesy flavors, which indicates that the oat flour may influence changes in constituents other than fat where an oxidation defect occurred. W.V.P.

256. Cooked Butter (Ghee). W. Ritter. Mitteilungen Lebensmitteluntersuchung und Hygiene, *28:* 206–14, 1937. (Conference held at the annual meeting of the Swiss Association of analytical chemists at Baden, June 11th, 1937.)

Pure butterfat is obtained by melting butter at about 40–50° C. and separating the fat from the aqueous parts, or by steam evaporation, which is the way of manufacturing ghee destined for sale in Switzerland.

Correctly cooked butter cannot develop bacterial defects, because the bacteria are lacking water, which is absolutely necessary for their propagation and vital activity. On the other hand cooked butter is more liable to oxidize than the original butter, because most of the elements which protect the fat against oxidation have gone into the cooking residue. However, the keeping quality of well cooked butter is by far better than that of butterfat melted at a low temperature. W.R.

CHEESE

257. Manufacture of Processed Swiss Cheese. C. R. Barker, Nat. Butter and Cheese J., *29:* 24, 20, 1938.

Specific instructions for the selection of cheese for blending and processing are given. Desirable forms of cheese are described and certain precautions are mentioned to avoid certain defects. W.V.P.

DISEASE

258. Double-Zone Beta-Hemolytic Streptococci. J. Howard Brown, J. Bact. *37:* 2, 133–144, 1939.

Double-zone beta-hemolytic streptococci which are found in the throats and vaginae of many normal persons are also the common cause of mastitis in cows. The organisms were found to fall into three fermentative groups and into Lancefield's serological group B. The recognition of the double-zone hemolytic streptococci is cited as a reason why diagnostic laboratories should not report all hemolytic streptococci as *Streptococcus hemolyticus.*

E.H.P.

259. Die Uebertragung der Bruzellosen zwischen den Tierarten und dem Menschen. (Transfer of Brucella infection between animals and man.) Alex Thomsen. Zsch. Infektkr. Haustiere. *52:* 4, 281–315.

This paper is mostly a review of the literature on Brucella infection in man and animals. No definite conclusion or recommendations are given.

The geographical distribution of the three types of Brucella infection is very wide and a variety of animal species are susceptible to infection. This paper includes an extensive survey of the subject together with a discussion of prophylactic measures to control the infection in animals and man.

L.D.B.

260. Differences in Immunization and Sensitization in Rabbits Injected with Relatively Avirulent or Highly Virulent Cultures of the same Strain (H) of Hemolytic Streptococcus. D. Murray Angevine, J. Exp. M. *69:* 2, 211, 1939.

A number of reports have previously shown that virulent organisms are usually more efficient in the production of immunity than are avirulent organisms. This worker, in a previous report, had made the observation that an avirulent hemolytic streptococcus (strain B_1) when injected repeatedly into the skin of rabbits produced a high degree of sensitization associated with only moderate immunity, whereas rabbits injected with a virulent hemolytic streptococcus (strain H) were only slightly sensitive but had conspicuous immunity. Because the variations in the immune response may have been due to the strains used rather than to the virulence of the organisms, a further study was made using the same strain of streptococcus both in an avirulent and in a highly virulent phase.

These studies showed:

When a relatively avirulent strain of hemolytic streptococcus was injected into the skin of rabbits a considerable amount of skin sensitivity to streptococcus filtrate developed, and there was scant demonstrable immunity.

When the same strain of streptococcus was made highly virulent by repeated passages through rabbits, little sensitization and considerable immunity was produced. C.N.S.

261. Detection of the Virus of Poliomyelitis in the Nose and Throat and Gastro-Intestinal Tract of Human Beings and Monkeys. S. D. Kramer, B. Hoskwith, and L. H. Grossman, J. Exp. Med. *69:* 1, 49.

The epidemiology of poliomyelitis strongly suggests the contact mode of infection. Experimental evidence furthermore points more specifically to the olfactory portion of the nasal mucous membrane as the portal of entry of the virus into the body.

Five strains of virus were recovered from nasal washings and feces. Four strains were of human origin, the fifth strain came from a monkey sacrificed at the height of the disease.

It is believed that the detection of the virus in the nasal washings of two additional patients during convalescence lends further support to the belief that the virus of poliomyelitis is spread by human contact.

The recovery of the virus from the feces 7 and 9 days after the onset of illness takes on added significance. It indicates first, that the virus withstands the gastric acidity which under normal physiological conditions tends to keep gastric contents relatively free of bacteria. It further suggests that improper disposal of feces from patients with poliomyelitis may have serious public health consequences, particularly in smaller communities where inadequate sewage disposal may result in contamination of surrounding beaches or even local water systems. C.N.S.

FOOD VALUE OF DAIRY PRODUCTS

262. Butterfat—Most Important Source of Vitamin A. James McLester. Am. Prod. Rev., *85:* 12, 308, 1938.

Four times the amount of Vitamin A required for absolute body needs is necessary for buoyant health. Butter yields 1,400 Sherman units per ounce. The daily minimum required for adults is 3,000 units more and is highly desirable. If butter alone furnishes the supply four tablespoons will provide the necessary amount. A pint of milk will supply 1,040 units, a dish of ice cream 670 units, and one ounce of cheese 700 units. The churning of butter does not cause a loss of Vitamin D. Storage of butter at low temperatures for as long as two years causes little, if any, loss of Vitamin A. P.S.L.

263. Dextrose in the Food Industries and Its Health Status. Carl R. Fellers, Massachusetts State College, Amherst, Mass., Am. J. Pub. Health *29:* 2, 135, 1939.

The commercial manufacture of dextrose and its properties and uses in the food industries are described. Legal and public health aspects are discussed. Nutritionally, dextrose and sucrose are equivalent. From a sanitary point of view, essentially no problems exist relative to the use of dextrose. M.W.Y.

264. Bread and Milk—An Ideal Food. James A. Tobey, Am. Prod. Rev. *85:* 12, 313, 1938.

Some 35 to 40 chemical substances are necessary for adequate nourishment of the human body. Bakers, who add milk solids to bread, have performed a real service to nutrition, for such bread will supply two-thirds of the necessary materials. Bread, while energy-producing, makes possible more complete utilization of the protective food, milk solids. Neither bread nor milk are fattening foods. Milk solids in white bread causes it to approach more nearly whole wheat bread in nutritional value. P.S.L.

265. Effect of Milk Supplement on the Physical Status of Institutional Children. III. Progress of Dental Caries. L. J. Roberts, S. Englebrecht, R. Blair, W. Williams and M. Scott. Am. J. Dis. Child. *56:* 805, 1938.

The effect of a milk supplement, with and without added Vitamin D, on the development of dental caries was observed for 90 children over a calendar year. The children were divided into three groups—one group remained as controls; the other two received daily per child a pint equivalent of evaporated milk, one having it in irradiated and the other in nonirradiated form.

All children showed extremely progressive caries. For the entire group there was a 26 per cent advance in number of carious teeth, a 50 per cent advance in number of cavities and a 98 per cent advance in the caries score.

The per cent age of increases in carious teeth was 28.7 for the control, 26.2 for the milk and 21.5 for the D milk group. The corresponding percentage of increase in number of cavities was 54.6, 46 and 50.7. These results indicate that the supplement tended to inhibit the rate of dental decay but that other factors also were involved. W.H.R.

HERD MANAGEMENT

266. Der Einfluss des Befruchtungszeitpunktes auf die Melk- und Trockenzeit. KALEFF, B. Zschr. f. Tierzücht. u. Zuchtungsbiol. *43* (1): 62–75, 1939.

Variations in service period (number of days from calving to the following conception) were studied in relation to milk production, to length of lactation after conception, and to length of the ensuing dry period, in 63 buffaloes, 146 cattle of the gray Bulgarian race, and 144 red cattle of mixed descent in the herd of the agricultural college at Sadowa. Some general observations on sheep are also included. Early rebreeding was most profitable. The weight of the newborn calves was not affected by the length of the preceding dry period nor by any circumstance other than extreme sickness of the dam. Cows kept for both work and milk conceived again sooner than those kept for milk alone. Some Bulgarian sheep produce lambs twice a year but others lamb only once. The difference is thought to be mainly in their heredity. Differences in regularity of calving among cows are also thought to be in considerable part hereditary and worth attention when selecting for breeding purposes. The general level of milk production in these herds was not high, the annual mean for the buffaloes being about 3,000 pounds. J.L.L.

ICE CREAM

267. The Institute Is Working for You. BERT SWEETING, Medosweet Dairies, Inc., Tacoma, Wash. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 7, Oct., 1938.

The activities of the Ice Cream Merchandising Institute are briefly reviewed. A series of merchandising short courses have been held in various cities in the country. A booklet entitled, "Let's sell Ice Cream" has been published. A testing laboratory has been set up in Washington for testing and perfecting new ways of serving ice cream at soda fountains. The two regular publications of the Institute, "Spinning Wheel" and "Ice Cream Currents," have been continued and improved. M.J.M.

268. **The A B C's of Selling.** Neal Bowman, Temple University, Philadelphia, Pa., Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 35, 1938.

The following definition for selling is given: Salesmanship is selling goods that will not come back to people who will come back. About 39 per cent of all the profit in merchandising is said to be lost on returned merchandise.

The author then illustrates each letter in the alphabet with a word representing some phase of selling, which he develops in an interesting manner to emphasize the important points in the psychology of salesmanship.

M.J.M.

269. **Don't Sell the Steak—Sell the Sizzle.** Elmer Wheeler, Tested Selling Institute, New York, N. Y. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 23, 1938.

This is an interesting presentation of the subject of, "What Makes People Buy?" The author discusses various tested methods which have been applied successfully to retail merchandising. M.J.M.

270. **The Ice Cream Industry's Opportunity in the Present Consumer Movement.** Miss Aubyn Chinn, The Borden Co., Chicago, Ill. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs. *4:* 11, Oct., 1938.

The author lists the principal professional and consumer groups of women and gives the approximate membership of each group. The present consumer movement had its inception in the depression. Every consumer group with any leadership is definitely taking part in influencing legislation.

The consumer wants business to take an interest in her wishes for information and in her buying problems. For self protection the ice cream industry should take advantage of this desire. The consumer is interested in having this information about any product: 1. Facts behind the manufacture of the product, 2. Quality, 3. Price, 4. Appearance, and 5. Care of the product.

There is no industry that has a more thrilling story to tell the consumer. The higher the quality the more zealous the ice cream manufacturer should be to encourage the consumer to inquire into the facts behind the product. There are all types of ice cream on the market today. Responsible manufacturers should encourage the discovery on the part of the consumer of those factors which make the difference between a good product at a fair price and a poor one. M.J.M

271. **Organization Building.** Irving C. Reynolds, Franklin Creamery Co., Toledo, Ohio. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 46, 1938.

Business has a very definite responsibility, which is to create and conserve human values to match its other achievements. In so doing it is necessary to develop and inspire leadership of sound character.

Certain incentives which have been found to be helpful are:—

1. Leadership—to be put in positions where they lead seems to develop greatly some people.
2. Promotion—looking forward to the realization of deferred payments benefits some workers.
3. Regular pay—with some it is the first consideration but not to others.
4. Commission or piece work—many people like to feel that their efforts are being measured.
5. Bonuses or prizes—as a rule stimulate effort for a short period of time.
6. Profit-sharing—a principle given great publicity but not very important in actual operation.
7. Power over things or over people—a stimulus to many.
8. Research or study—many people have a craving to develop themselves in their industry.
9. The family spirit—the feeling of unity in an attempt to suceed invaluable.
10. Unionism—this means a great deal to many people and the principles of the strength of the union cannot safely be overlooked.

M.J.M.

272. How to Use Color in Ice Cream Merchandising and Advertising. W. A. Sumner, Univ. of Wisconsin, Madison, Wis. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 53, 1938.

The right colors should be used on delivery trucks, on cartons, in advertising and in the product itself.

In the advertising of food products we should choose colors which suggest "cleanliness." We want to use colors always that are clean. The lighter shades, white, and light shades of yellow and ivory, light green, and light blue, all suggest cleanliness.

In the proper use of color get a good artist who has good taste and then do not insist on cluttering his design of that carton or other design with too much material to compete with it, in terms of letters and lettering.

M.J.M.

273. Ice Cream Merchandising—Its Possibilities. F. D. Mayer, Pres., Merchandising Institute of America, Chicago, Ill. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 60, 1938.

The importance of introducing an original idea in selling which will motivate people to buy is essential for satisfactory results. A buying motive which, psychologically, appeals to many should be created. Various ideas are cited which have been very successful in other industries and the reasons for their success is discussed. M.J.M.

274. The Composition and Serving Temperature as a Means of Increasing Consumer Preference for Ice Cream. W. H. E. Reid, R. J. Drew, and W. S. Arbuckle, Univ. of Missouri, Columbia, Mo. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 76, 1938.

The authors draw the following conclusions from their experiments:

1. Ice cream containing 14 per cent fat was given the highest consumer preference at ordinary serving temperatures. However, at high serving temperatures, a fat content of 16 per cent seemed most acceptable and at very low serving temperature, 12 per cent fat was preferred.
2. Ice creams containing 13 per cent serum solids had the highest consumer acceptance at serving temperatures of 10° F.
3. With an average serum solids content of 10 per cent, 16 per cent sugar was preferred to other amounts of sugar. However, when the serum solids content was as high as 13.5 per cent, then 14 per cent of sugar was sufficient.
4. Both the composition and serving temperature affect directly the number of dishes of ice cream obtained to the gallon.
5. With increases in either fat or gelatin, the ice creams became more stable at room temperature. The serving temperature is of little significance when the fat or serum solids content is high. However, with low fat and low stabilizer percentages both the serving temperature and the sugar content are of considerable significance.

The results of these investigations indicate definitely that the composition and serving temperature of ice cream are factors that have a decided effect upon consumer acceptance, and that the manufacturers and dispensers may regard them with considerable importance, as a means of increasing consumer preference of their product. M.J.M.

275. Ice Cream Can Analysis. Special Bulletin No. 59. General Survey of the Int. Assn. of Ice Cream Mfrs., 1105 Barr Bldg., Washington, D. C.

The survey of 470 plants making 88,800,000 gallons of bulk ice cream, shows that 46.2 per cent of the ice cream companies reporting use steel cans exclusively while only 8.1 per cent use paper cans entirely. However, small companies use the steel cans and large companies use paper cans. Therefore, a much greater amount of ice cream is packed in paper than the above figures would indicate. Some manufacturers (45.7 per cent) use both paper and steel cans and give the customer the choice. Others use the steel cans as long as they are usable and gradually shift to paper as the steel cans need to be discarded. The 5 gal. size of steel can is used more than the 2½ gal. size but with paper cans the reverse is true.

So far as plant costs are concerned, in the plant only, the cost of the steel cans is less than that of paper. However, the use of paper cans decreases the trucking weight about 30 per cent. In rail shipment of ice cream a very considerable saving in shipping costs can be made by the use of paper cans instead of steel cans. M.J.M.

276. **Preliminary Report on Ice Cream Can Survey.** O'NEAL M. JOHNSON, Washington, D. C., Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 12, Oct., 1938.

Editors Note: This is a report of the data obtained in the survey reported in Special Bulletin No. 59 and abstracted in the previous abstract.

277. **Contamination of Fountain Utensils.** ANONYMOUS. Ice Cream Rev., *22:* 8, 94, 1934.

The great majority of ice cream outlets continue to operate with dirty dippers in a manner that leaves the entire ice cream industry open to the threat of investigation by public health authorities and a flood of unfavorable and injurious publicity. J.H.E.

278. **Some Causes of High Power Costs.** C. T. BAKER, Consulting Engineer, Atlanta, Ga. Ice Cream Rev., *22:* 8, 88, 1939.

In many cases investigated it has been found that the major factor influencing power cost in ice cream factories has been high condensing pressures. Suggestions for reducing condenser pressures are given. J.H.E.

279. **Storage of Cream for Ice Cream.** J. J. SAMPEY. Abbott's Ice Cream Corp., Philadelphia, Pa. Ice Cream Rev., *22:* 8, 78, 1939.

Since copper contamination accelerates the development of an oxidized flavor in frozen cream during storage it is desirable to determine the amount of copper which cream may have picked up during processing. Several methods proposed for the determination of copper in milk products are discussed. J.H.E.

Editors Note: This article is essentially the same as that of abstract No. 280.

280. **Storage of Cream for Ice Cream.** J. J. SAMPEY, Abbott's Dairies Inc., Philadelphia, Pa., Ice Cream Trade J., *35:* 3, 40, 1939.

The suitability of various tests which are available for the selection of cream that can be stored satisfactorily without the development of off flavor is discussed.

A test for the determination of copper reported by Ritter in 1936 was described which showed fair agreement with other methods, however it is not satisfactory for high acid cream.

Ten cc. of cream are heated in a test tube to 195° F. by immersion for two to three minutes in a bath of boiling water. (This is to insure freedom from disturbance by bacteria.)

To the heated and cooled sample is added one drop of:

(a) para-amino dimethyl anilin sulphate prepared by dissolving 0.2 gram in 5 cc. water, boiling with bone charcoal and filtering. This is not stable for more than an hour or two.

(b) alcoholic alpha-naphthol, strength not important (5 per cent works well).

(c) 1 per cent hydrogen peroxide.

The exact amount of (a) and (b) is not critical, but the amount of peroxide is.

The sample is then held at incubation temperature and observed for formation of a blue color.

If much copper is present, this will be formed in a few minutes, and if little, three or four hours.

Evaluation of the amount present may be made by performing the test on samples of known freedom from copper as well as on those with definite amounts added. It is stated that the test is sensitive to 1/20 part of copper per million.

Another test reported by Hileman & Webb J. D. S. xx: 47, 1937, seems more applicable to cream.

This depends on the fact that milk or cream contaminated with copper during pasteurization or processing will show an increase in oxidation reduction potential. If the Eh. of the original milk or cream is first determined and the Eh. of the final product be shown to have a rise of Eh. 100 to Eh. 200, such cream should be considered as unsuitable for storage purposes and should be used within a few days of its production.

This method is particularly useful in tracing copper contamination in the flow of milk or cream through plant equipment. W.H.M.

281. Getting that Fresh Fruit Flavor. D. G. SORBER. Food Research Div., U. S. D. A., Ice Cream Rev., *22:* 8, 50, 1939.

The natural fresh fruit flavor and color of fully ripened fruit can be preserved to a very high degree by a new method of preparation and preservation worked out by the U. S. Laboratory of Fruit and Vegetable Chemistry. The method consists of reducing the fruit to a pulp or puree, adding a predetermined amount of sugar or sirup, packaging in tight containers, and freezing rapidly enough to prevent deterioration. A storage temperature of 0° F. is recommended. J.H.E.

282. Merchandising Ice Cream through the Retail Store. HAROLD GOSSER. Bard's Dairy Stores, Pittsburgh, Pa. Ice Cream Rev., *22:* 8, 46, 1939.

This article contains many helpful suggestions for the successful operation of a retail ice cream store. The three most neglected details in store operation are said to be; dirt, stale, skimpy goods, and lack of friendly interest. In these days most employees at the start are not only unskilled and immature but aso have very little idea of discipline, workmanship, or courtesy. Since no one else has done this teaching the operator of stores must follow a well planned personnel program. J.H.E.

283. **Ice Cream Cabinet Maintenance.** ANONYMOUS. Ice Cream Rev., *22:* 8, 38, 1939.

Directions are outlined for the care and upkeep of electric motors on ice cream cabinets. J.H.E.

284. **Save Money by Refining Raw Sugar.** ANONYMOUS. Ice Cream Rev., *22:* 7, 56, 1939.

A newly developed refining unit makes it possible for ice cream manufacturers to refine raw sugar into a clear white sugar syrup for use in ice cream. A saving in cost per pound of sugar solids is claimed. J.H.E.

285. **Preserving the Freshness of Ice Cream.** A. D. BURKE AND R. M. NEWMAN. Ala. Poly. Inst., Auburn, Ala. Ice Cream Rev., *22:* 7, 50, 1939.

Oat flour (Avenex) added to normal ice cream mix at the rate of .5 per cent retarded the development of a stale flavor upon storage. The melting properties of ice cream made from butter as the sole source of fat were improved by the addition of the oat flour. J.H.E.

286. **Protection of Ice Cream Against Contamination after Pasteurization.** F. E. NELSON, Kans. Agr. Expt. Sta., Manhattan, Kans. Ice Cream Rev., *22:* 7, 46, 1939.

Results indicate that if organisms of the Escherichia-aerobacter group are present in a sample of ice cream, negative to the phosphatase test, they are indicative of contamination following pasteurization. Use of these two determinations in locating sources of contamination in ice cream plants is suggested. J.H.E.

287. **Sterile Glassware at the Soda Fountain.** ANONYMOUS. Ice Cream Rev., *22:* 7, 42, 1939.

A description is given of a new ultra-violet light sterilizing cabinet for soda fountain glassware. In tests made the bacterial reduction of *E. coli* and *B. subtilis* was 99 per cent. J.H.E.

288. What's the Outlook for Specialized Ice Cream Stores? ANONYMOUS. Ice Cream Rev., *22:* 7, 68, 1939.

Present trend in size and type of retail outlets is away from specialization and toward a return to the old general store concept. J.H.E.

289. How Can Plant Operating Efficiency Be Increased? H. J. BROWN, Goldenrod Ice Cream Co., Chicago, Ill. Ice Cream Rev., *62:* 7, 62, 1939.

Suggestions are given for the control of plant operations leading to greater efficiency and reduced production costs. J.H.E.

290. Practical Savings in Accounting Office Costs—Round Table Discussion. J. S. BLOOM, Abbott's Dairies, Inc., Philadelphia, Pa. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 64, Oct., 1938.

Points stressed in the discussion were the choice of personnel, flow of work, light and air for office workers, office equipment, usefulness of reports and employee relations. M.J.M.

291. Report of Statistical and Accounting Bureau for 1937–38. O'NEAL M. JOHNSON, Washington, D. C. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 61, Oct., 1938.

A tabulation is given of the number of companies and gallonage represented in the expense comparisons of the association. Special surveys made during the year included the retail store analysis, the ice cream can survey, and the labor analysis. The ice cream sales index was also determined. Through August of 1938, a decrease in gallonage of 4.98 per cent was found for the United States, and for Canada, 4.66 per cent. M.J.M.

292. The Influence of New Products on Costs. H. C. THOMAS, Harrington & Company, Dushore Pa. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 55, Oct., 1938.

From 1930 to 1935 the sale of packaged ice cream increased about 50 per cent. The need for getting actual costs on the various products sold is more essential now than ever before. The procedure used by Mr. Thomas was to set up a "Brick and Specialty Department" and charge against the products made in it all necessary costs, as fixed charges, labor, repairs, supplies, etc. The products costs, refrigeration costs, delivery and sales costs, are all charged in direct proportion against this department. In this way the influence of any new product on costs is easily determined. M.J.M.

293. The Use of Product Costs by Management. C. A. ARMITAGE, United Farmers Cooperative Creamery Assn., Charlestown, Mass.

Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 45, Oct., 1938.

The accounting division should prepare "Product Cost" figures accurately and promptly for each month or quarter. The production department has the benefit of comparisons between current costs and those of previous periods, and should work closely with the sales department in an effort to produce at costs which will permit a satisfactory gross profit to be realized. Close cooperation of all departments in the use of "Product Costs" should react to the benefit of the entire business. M.J.M.

294. Practical Savings through the Use of Accounting. P. B. BECK, The Borden Company, New York, N. Y. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 35, Oct., 1938.

The cost of accounting must be met by saving from accounting. Therefore, only those records which are indispensable should be kept.

Detailed expenses as to total and unit cost per gallon for each month must be known. Departmental and divisional costs must also be known. If the average costs are known, then savings can be made when certain cost figures are evidently too high. Suggestions are made for savings in water, power and refrigeration costs. Other preventable costs cited as examples are those for preventable accidents, fires and unnecessary telephone and telegraph expenditures. M.J.M.

295. Eliminating Cost Distortions Due to Seasonal Volume Variations. L. C. ANDERSON, General Ice Cream Corp., Schenectady, N. Y., Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 25, Oct., 1938.

An accountant should furnish the operating executives, at least monthly, data on the following points:

1. Payroll comparisons, 2. Expense comparisons, 3. Data on volume and price trends, 4. Cost of mix materials, 5, Data on yields and losses, and 6. Data on route operations.

Current data sufficiently reliable to inform the management as to these trends should be sufficient for management to guide operations intelligently. Further data of a non-current nature is also necessary, such as information as to selling prices needed for profitable operations and which items produce the most profits. If different plants are operated, the relative efficiency of the different units must be determined. M.J.M.

296. President's Address. J. L. BLOOM, Abbott's Dairies, Inc., Philadelphia, Pa. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 7, Oct., 1938.

This address is a discussion of the tendency towards increased governmental regulation of business. The suggestion is made that companies should maintain satisfactory public relations so as to avoid undue difficulties in dealing with employees and consumers. M.J.M.

297. Nutritional and Health Values of Frozen Desserts.

W. H. E. Reid, Dairy Dept., Univ. of Mo., Ice Cream Trade J., *35:* 2, 20, 1939.

Attention is directed to the composition of ice cream and the function of the various constituents from a nutritional standpoint. Emphasis is placed on the care exercised in the manufacture of factory made ice cream and on the sanitary quality of the product which results from inspection and supervision of the manufacturing processes. Testimonials from nutrition authorities as to the merits of ice cream as a food are cited.

According to these findings, ice cream possesses the most desirable eating qualities from the standpoint of flavor, body, and texture when served at a temperature of 12 to 14° F. W.H.M.

298. Controlling Oxidized Flavors.

A. C. Maach and P. H. Tracy, Dairy Ind., Univ. of Ill., Ice Cream Trade J., *35:* 3, 32, 1939.

The protective action of oat flour (Avenex) in preventing oxidized flavor development in ice cream, sherbets and sweet cream and the effect of the flour on other properties of the ice cream were studied. Results reported by the authors indicate: 1, When added at the rate of 0.3 to 0.5 per cent to vanilla ice cream mix containing 3 p.p.m. copper before freezing, Avenex seemed to protect the ice cream from a stale flavor for two weeks. 2, In strawberry ice cream the protective action of the oat flour was not as great as in vanilla ice cream. Samples which contained 2 and 3 p.p.m. copper were metallic after the first week and more so after two weeks. Samples containing 1 p.p.m. copper were slightly metallic at the end of two weeks. 3, In strawberry sherbet oat flour was reported to be helpful in flavor and body improvement. 4, Oat flour added to sweet cream at the rate of 1.5 per cent in the form of a gruel before pasteurization afforded rather good protection to the cream stored in the frozen state. Dry oat flour added to this cold cream was of no value. 5, Oat flour when added at the rate of .5 per cent to the ice cream mix had no effect on titratable acidity or pH of the mix, doubled the viscosity over that of the control, did not effect the melt down properties of the ice cream and did not appreciably affect the time required to get 100 per cent overrun. Difficulty was reported in making sediment tests on mixes containing oat flour. W.H.M.

MILK

299. The Influence of Heavy Metals on the Enzymic Reactions of Milk.

W. Ritter, Fifth International Congress for Technique and Chemistry of Agricultural Industries, Scheveningen, *2:* 297–306, 1937.

The influence of copper and iron on the aldehyde-reductase (xanthine-oxidase) of milk and cream, as well as the occurrence of positive peroxidase reactions in heated milk and cream and their influence on the oxidation constancy of milk are treated in this paper. Small amounts of copper are sufficient to prolong very much the methylene blue reduction time of a mixture of milk and formaldehyde. All copper containing alloys (brass, bronze, German silver, monel metal) produce the same effect, though in a somewhat lesser degree than pure copper.

In just the same way the presence of small amounts of copper is able to develop a positive peroxidase reaction in milk. The effect of copper on this reaction works about parallel with its effect on the flavor of the milk, which becomes tallowy. Nickel and iron also produce a bad flavor in the milk, but they do not alter its enzymic reactions. W.R.

300. The Influence of Pasteurization-Temperature on Certain Properties of Milk.

W. Ritter. Schweiz. Milchzeitung 1937 (special number published in honor of the 70th birthday of Prof. Dr. Rob. Burri).

Besides the well-known effects of increased temperature on milk (destruction of bacteria and of enzymes, coagulation of the albumin, alteration of the composition of milk-salts, of cream formation, of color, smell and flavor and so on), heating also influences to a great extent the reaction of milk in oxidation and reduction processes. The following points are to be taken into account: According to investigations of Burri and Kursteiner of 25 years ago, milk submitted to high heating is able to reduce methylene blue under exclusion of oxygen. This effect is perfectly independent of bacterial and enzymic activity. Differences occur in determining the reduction time of bacteria cultures inoculated on milk heated at lower temperature, for instance such as has been submitted to holding pasteurization or to a temperature of 80 or 90° C. Methylene blue is more rapidly reduced in milk heated at high temperature than in milk submitted to holding pasteurization. On the other hand, the influence of copper on the reappearance of the color on shaking the samples is more perceptible in milk submitted to holding pasteurization than in milk heated at 80 or 90° C. Peroxydase- and nadi-reaction on milk heated at high temperature, if founded on purely chemical reasons (presence of small amounts of copper), is all the more feeble as the heating has been more intense or longer. This dependence becomes also evident when a small amount of raw milk is added to heated milk. The effect of copper and iron on the developing of tallowy flavor in milk heated at different degrees is analogous.

Milk that is liable to become tallowy shows the preventive or at least checking effect of intense heating on this defect. These questions are of great importance for condensed milk and milkpowder, which also reveal different constancy against oxidation, according to the degree of heating; this circumstance may lead to the abolition of the still frequent use of copper vacuum apparatus. When butter is prepared with acid cream, the important fact appears that the heating of the cream at 90° C. in flash-pasteurizers prevents the developing of fishy flavor during the storing, while heating at 80–84° C. is not sufficient for that effect. Butterfat obtained at low temperature is much less constant against oxidation than properly cooked butter. W.R.

301. Off-Flavors in Winter Milk. H. J. Brueckner, Am. Pro. Rev. 85: *14*, 378–79, 1938.

The period of year during which cows are stabled is the period during which disagreeable flavors occur most frequently in milk. Feeds imparting off flavors should be fed only after milking. Most grain mixtures may be fed during milking. Air impregnated with feed odors and breathed by the cow may cause transference of the odor to milk before it is drawn. Ventilation will remove much of this difficulty.

A common abnormal flavor occurring especially in raw milk and cream is that of bitterness or rancidity. This develops apparently more frequently with cows late in the lactation period and especially with those in poor condition through underfeeding and with those who are low producers. In extreme cases the flavor develops in four to six hours, but usually 12 or more hours are required. The defect is due to the action of the enzyme, lipase. Tests for amounts of chlorides present will give a clue to whether the milk will become rancid but if mastitis is present the test interpretation is misleading. Flash heating of milk to 120°–130° F., cooling to 75–85° F. before separation to partially restore viscosity will aid in prevention of the flavor in cream. Pasteurization before the flavor develops will prevent the flavor in milk. This process will not eliminate the flavor once it is present.

Oxidized or tallowy flavor is chiefly caused by passing warm or hot milk at the farm or factory over copper, iron, copper alloys as bronze, brass, ambrac, or German or nickel silver. The only remedy seems to be the elimination of any equipment containing copper in the surface exposed to milk. Other factors, less easily controlled, are the greater susceptibility of some milk to develope the defect, exposure of milk to sunlight, chlorine sterilization of utensils, and the amount of air in milk. P.S.L.

302. Revision of Methods and Standards for Certified Milk. J. Howard Brown, Johns Hopkins Univ., Baltimore, Md. Chairman, Committee on Methods and Standards of the American Association of Medical Milk Commissions, Am. J. Pub. Health, *29:* 4, 355, 1939.

Topics discussed are the bacterial plate count using an improved medium, coliform counts and their significance, the spore test, identification of hemolytic streptococci and other tests. The author urges health officials to take a broader interest in milk than to make it safe and points out that there are possibilities of improving the flavor and nutritive quality of milk.

M.W.Y.

303. Place of the Methylene Blue and Resazurin Reduction Tests in a Milk Control Program.

C. K. Johns, Dominion Dept. of Agr., Ottawa, Ontario, Canada. Am. J. Public Health *29:* 3, 239, 1939.

In routine testing of incoming supplies, the author believes that the methylene blue reduction test has many advantages over the usual counting methods. Inversion of tubes every two hours yielded results in good agreement with those from more frequent mixing and greatly improved the accuracy of the test as compared to the standard method. The resazurin one-hour test did not indicate the bacterial content as reliably as did the modified methylene blue reduction test. M.W.Y.

304. Sanitary Dispensing of Beverages.

Editorial, Am. J. Pub. Health, *29:* 2, 166, 1939.

Devices have been perfected for the hygienic dispensing of pasteurized milk from milk containers in restaurants. After more than three years' study, seven such devices have been approved by the Health Department of New York City. The essential features are simplicity and ease of operation. They should be made of materials that not only resist contamination and are non-toxic, but are so constructed that they may be frequently and easily disassembled and quickly and effectively cleansed or sterilized in boiling water or appropriate chemical solutions. M.W.Y.

305. Value of Phosphatase as an Indicator of Pasteurization.

F. W. Gilcreas, Div. of Laboratories and Res., N. Y. State Dept. of Health, Albany, N. Y. Am. J. Pub. Health, *29:* 2, 158, 1939.

The phosphatase test gives precise information concerning the pasteurization of milk. Prolonged preheating or slight over-heating may reduce the phosphatase content of the milk sufficiently to make it impossible for the laboratory test to detect technical shortening of the holding time by 5 minutes or the addition of 0.1 per cent of raw milk.

Cream separated from raw milk and then pasteurized gives a reaction of complete treatment. Cream separated from pasteurized milk either by gravity or by mechanical means tends to have a high phenol value indicative of under-pasteurization. The tendency for the phosphatase value of cream to increase beyond that of the original milk is discussed, and the statement made that it may become necessary to establish a separate code of readings for cream. M.W.Y.

MISCELLANEOUS

306. The Influences of Cleaning Materials on some Metals. W. Ritter and Ths. Nussbaumer: Schweiz. Milchzeitung No. 51, 53, 55, 56, 57, 1937.

It was found that no perceptible corrosion of the metals employed in dairy industry is produced by the commonly used cleaning materials "Neomoscan," "P_3-zinnfest" (which does not attack tin) and "P_3-sterile." On the other hand the material called "Try" which coontains trisodium-phosphate effects a strong corrosion on aluminium and its alloys on account of its too low sodium silicate content. The milk-stone solvent "Tartrex" which consists mainly of winestone (acid potassium-tartrate) distinctly attacked tinned metals, while it did not produce much effect on aluminium and its alloys. On examining metal combinations in conducting connection, we obtained weak currents when dissolvents containing sodium silicate were used and rather strong currents with soda alone. The latter currents produced accordingly strong corrosions. Relatively strong currents which scarcely slackened after 24 hours resulted when metals in conducting connection were immersed in Tartrex solutions. A stronger corrosion of the baser metals was quite apparent, while the more precious metals were less attacked in corresponding experiments with single metals. Conditions were particularly marked in a combination of copper and aluminium. While tinned material was distinctly attacked by Tartrex alone, the corrosion was still stronger in tinned material in conducting connection with stainless steel. Tinned material proved baser at first in Tartrex solutions than aluminium, but the direction of the current changed after a few hours. None of the tested cleaning materials produced a perceptible corrosion on steel alone or combined with other metals. Anode oxidized aluminium and its alloys were attacked if the sodium silicate content of the solutions was too low. W.R.

307. Some Properties of Lactose. W. Ritter: Schweiz, Milchzeitung No. 71–74, 1937.

Properties, formation and transformations of the three well-known forms of lactose (α-lactose-hydrate, α-lactose-anhydride and β-lactose-anhydride) are discussed and their importance for dairy problems (sandiness in sweetened condensed milk and ice cream, action in milk powder) is made known. It follows, from the conditions of formation, that the lactose in the cooking residue of butter is certainly present in the form of β-lactose-anhydride.

W.R.

PHYSIOLOGY

308. Roentgen Studies of the Mechanism Involved in Sperm Transportation in the Female Rabbit. Robert H. Krehbiel and Herman

P. CARSTENS, Dept. of Anatomy and Physiology, Univ. of Ill. Am. J. Physiol. *125*, 3, 571, 1939.

Transfer of methylene blue, janus green B and iodochlorol through the reproductive system of oestrus rabbits have been observed after artificial stimulation of the vulva. These dyes reach the tubal end of the uterine horns 2 to 5 minutes after the start of stimulation. The observed transfer of fluids into and through the uterus may be interpreted as being similar to the transfer of spermatozoa and would indicate that spermatozoa are passive in their movement to the utero-tubal junction in animals which have an extended heat period. Despite the continued activity on the part of the vagina and uterus over a period of an hour the authors were unable to detect any fluid in the tubes. In fact, pressures were created at the junctions of the uterus and tubes which would be sufficient to force the liquid into the tubes if a sphincter were not present. D.L.E.

309. Gonadotropic Extracts from Green Leaves.

MAURICE H. FRIEDMAN AND GERTRUDE S. FRIEDMAN, Dept. of Physiology, Univ. of Pennsylvania Medical School. Amer. J. Physiol. *125*, 3, 486, 1939.

Because of the protein nature of the pituitary hormones and the fact that qualitative or quantitative restriction of dietary protein is known to interfere with reproduction, the authors decided to test the hypothesis that dietary proteins contain indispensable precursors for the formation of the pituitary proteins. They found that it is possible to extract from the dried leaves of immature grasses a fraction which is capable of producing ovulation upon injection into rabbits. The observed chemical properties of this fraction simulate the properties of the gonadotropic material in anterior pituitary extracts. D.L.E.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

310. Determination of the Heat Resistance of Non-spore-forming Bacteria.

E. E. BAKER AND L. S. MCCLUNG, Division of Fruit Products, Univ. of California, Berkeley, Calif. Food Research *4:* 1, 21, 1939.

In this comparative study of technics for determining the heat resistance of non-spore-forming bacteria, evidence is presented showing the necessity for close spacing of heating periods, for long heating periods and for the heating of several tubes at each heating period. It is stated that much of the earlier work on this subject can be criticized because of failure to observe these essential points of technics. F.J.D.

BUTTER

311. Effect of Variation in Churning Acidity on Manufacture of Butter.

E. W. BIRD, Dept. of Dairy Ind., Iowa State College, Ames, Iowa. Nat. Butter and Cheese J., *30:* 1, 16, 1939.

The effects of acidity on cream constituents during churning can be attributed to the changes that occur in the casein. Fat losses tend to be lowest when the fresh butter serum has a pH of 6.5 to 7 and when titratable acidity of the neutralized cream approximates 0.16 to 0.18 per cent.

Approximate pH values of butter serum associated with flavor defects are:—above 7.5—alkaline or neutralized; 6.8 to 7.4—least objectionable flavors with "old cream" predominating; below 6.8—old cream, high acid and sour, show and become more pronounced; below 6.6—metallic flavors; 5.8 to 6.2—cheesy; below 6.0—fishy. W.V.P.

312. Les Phosphatides dans le Résidu du Beurre. (The Phosphatides in the Residue of Butter.)

B. REWALD. Le Lait, *19:* 22–25, 1939.

Pure butter oil is produced in some European countries. In this process, the moisture is driven off and a residue settles out. The fatty materials can be extracted from this by means of petroleum ether and acetone. The extract is a mixture of lecethin (soluble in alcohol) and cephalin (insoluble in alcohol) as well as some other material. All the phosphatide extracts present are of the monoamino-monophosphatide type having a P/N ratio of approximately 1/1. O.R.I.

313. The Phosphatase Test—Its Application to Sour Cream and Sour Cream Butter.

E. H. PARFITT AND W. H. BROWN, Purdue University, Lafayette, Ind. Nat. Butter and Cheese J., *30:* 1, 12, 1939.

The phosphatase test is in general a good tool to use to control the pasteurization of cream for buttermaking and to detect butter made from inadequately pasteurized cream. Application of this test to sour cream and sour cream butter to determine adequate pasteurization is more complex than its application to sweet products. Some of the factors which complicate the procedure are: variations in the concentration of enzyme in cream; the inactivation of the enzyme with time and temperature of storage; the insulating action of milk fat; the existence of phenol-producing bacteria; the possibility of phenol in the wash water; the use of chlorinated wash water; the activation of the enzyme in butter during storage; and the concentration of the enzyme in the buttermaking process. W.V.P.

314. How Butter Consumption Varies. MARVIN A. SCHAARS, Univ. of Wisconsin, Madison, Wis. Nat. Butter and Cheese J., *30:* 3, 8, 1939.

Various studies show that butter consumption per capita varies with nationality, race, number of adults and number of children in a family. High consumption per capita is associated with high income, small families, families of adults only and with Americans or native whites; low consumption with low income, large families, families with children, and the Negroes and people, like Italians, who use oil in place of butter for cooking. Annual per capita consumption in the United States for 1937 was 16.7 pounds. A survey of the white population is cited which shows the annual per capita consumption by regions of the United States to approximate: N. Atlantic, 21 lbs.; E. N. Central, 15.3 lbs.; E. S. Central, 18.4 lbs.; Pacific, 22.1 lbs. Negroes of South average about 6.6 lbs. per person per year. W.V.P.

315. Making America Safe for Butter or Worse. H. A. BENDIXEN, State College of Washington, Pullman, Wash. Nat. Butter and Cheese J., *30:* 3, 10, 1939.

In order to keep the consumer buying butter it is necessary to advertise its food value, flavor, and healthfulness. Consumer desires in flavor, body, salt content and package should be studied. Selling by some government established grade will stimulate consumer confidence. Some advice on controlling butter quality is given. W.V.P.

316. Dichlorofluorescein to Estimate Salt Content of Butter. F. M. SHELTON AND L. R. BRYANT. Nat. Butter and Cheese J., *30:* 4, 16, 1939.

Dichlorofluorescein, an adsorption indicator, produces a distinct and sudden color change end point when a salt solution is titrated to neutrality with silver nitrate. Dichlorofluorescein and potassium chromate were com-

pared as indicators in analyzing butter for salt. The new indicator checks very favorably with potassium chromate and gives more uniform results.
W.V.P.

317. A Study of Roll-less Churns. C. H. PARSONS, Swift & Co., Chicago, Ill. Nat. Butter and Cheese J., *30:* 5, 12, 1939.

In the new types of roll-less churns churning time is shortened, residual butter fat in the buttermilk is no higher, working time is the same, the butter contains less air, and moisture and salt are more uniformly distributed. The new-type churn is more flexible in capacity and is decidedly more sanitary than the old type. It is hazardous to draw conclusions on such new equipment but the roll-less churn at present will justify unbiased investigation. W.V.P.

CHEESE

318. Determining Salt Content of Cheese. O. R. OVERMAN AND BURTON F. WHITMORE, Univ. of Illinois, Urbana, Ill. Nat. Butter and Cheese J., *30:* 2, 18, 1939.

A procedure is given for measuring salt in cheese by digesting the cheese in a side arm distilling flask with strong (35 per cent) sulfuric acid and absorbing the liberated hydrogen chloride in a measured volume of a standard solution of silver nitrate. The method is simple, short and checks closely with the official method. W.V.P.

319. Le Dosage de la Matière Grasse dans les Fromages. (The Determination of Fat in Cheese.) D. FLORENTIN. Le Lait, *19:* 25–29, 1939.

In an examination of three methods for determining fat in cheese the use of the Soxhlet extraction method was found to give higher values than did other methods in which the cheese was first digested with concentrated hydrochloric acid. Digestion with acid was shown to hydrolize the fat to some extent and the glycerol and soluble fatty acids thus produced were lost in the washing which preceded the final ether extraction. O.R.I.

320. Proposed Changes in Scoring Cheddar Cheese. J. C. MARQUARDT, New York Agr. Exp. Sta., Geneva, New York. Nat. Butter and Cheese J., *30:* 4, 12, 1939.

Cheese flavor should be added to the score card and designated as "lacking," "mild," "good," "clean," or "strong"; or, these characteristics should be given a numerical rating of 50, 60, 75, and 95 respectively. It is proposed to reduce score for finish from 15 to 10 points and to use the five points to describe salt content, as determined by chemical analysis.
W.V.P.

321. Suggested Standards for the Cheese Industry. RAYMOND MIOLLIS, Natural Cheese, Inc., Chicago, Ill. Nat. Butter and Cheese J., *30:* 3, 64, 1939.

Laws controlling cheese composition should be simplified by defining varieties by classification, specifications and nomenclature rather than by percentage of fat and moisture. The manufacture of raw-milk cheese should be prohibited and strict rules should regulate cheese processing.

W.V.P.

322. A Vat for Experimental Cheese. C. B. LANE, Iowa Agr. Exp. Sta., Ames, Iowa. Nat. Butter and Cheese J., *30:* 2, 32, 1939.

Specifications are presented for the construction of a multiple cheese vat in which 20 lb. lots of milk can be given identical curd making treatments.

W.V.P.

323. Flavor Contributors of Swiss-Type Cheese. F. J. BABEL AND B. W. HAMMER, Iowa Agr. Exp. Sta., Ames, Iowa. Food Research, *4:* 1, 81, 1939.

Swiss-type cheese with considerable of the characteristic sweet flavor yielded a much higher volatile acidity on steam distillation than cheese lacking in flavor. Added calcium or sodium propionate gave to process Swiss-type cheese a sweet flavor and a texture and color more like natural Swiss cheese. The results obtained suggest that propionates are important flavor contributors of Swiss type cheese. J.F.D.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

324. Fluorosis in Rats Due to Contamination with Fluorine of Commercial Casein. The Effects of Darkness and of Controlled Radiation upon the Pathology of the Teeth. HAROLD C. HODGE, ETHEL M. LUCE-CLAUSEN, AND ELIZABETH F. BROWN, Univ. of Rochester, Rochester, N. Y. J. Nutrit., *17:* 333–346, 1939.

Rats which received a diet containing commercial casein contaminated with fluorine manifested the usual tooth changes associated with fluorosis. The casein contained 0.2 per cent fluorine while the diet contained only .03 per cent. Spectrograph analysis of both commercial and purified casein obtained from several different companies showed them free of fluorine.

C.F.H.

325. Nouvelles Études sur le Dosage des Matières Minérales des Caséines Lactique. (New Studies on the Amount of Ash in Lactic Acid Casein.) JEAN PIEN AND M. WEISSMANN. Le Lait, *19:* 15–22, 1939.

Methods for the ashing of casein which will retain the organic P_2O_5 are discussed. The authors suggest the use of five grams of ground casein as a sample plus 5 ml. of a 10 per cent solution of magnesium acetate in order that the organic P_2O_5 may not be lost in the ashing. The addition of H_2SO_4 to facilitate the ashing of casein is condemned. O.R.I.

DISEASE

326. A Comparison of Cultural Methods in the Diagnosis of Bovine Mastitis.

T. L. JONES AND F. W. SCHOFIELD, Dept. of Bact., Ont. Veterinary Coll., Guelph, Ont. Canad. Pub. Health J., *30:* 46, 1939.

Attempts were made to simplify several of the more reliable, but time-consuming tests for mastitis. A comparison of the growth produced from centrifuged cream layers as against the centrifuged sediment showed that the former was unreliable. Experiments were also made to determine whether or not the addition of the dye in the Hotis and Miller test inhibited bacterial growth. While no evidence of inhibition was obtained, the authors suggested that the dye be added 5 to 10 hours after incubation has begun. O.R.I.

327. Observations on a New Test for the Presence of Mastitis in Milk.

W. H. WHITESIDE, Kennedy Biological Laboratories, Otterville, Ont. Canad. Pub. Health J., *30:* 44, 1939.

It was found that the addition of 2 cc. N.NaOH to 10 cc. of milk of cows suffering from mastitis produced a viscid mass when stirred with a glass rod. The test fails if the mixture is brought to the boiling point.

This test becomes positive before the brom-thymol blue and strip-cup tests. It is invariably negative with the milk of uninfected cows.

If the mixture is incubated at 37° C., it develops a bright cherry red color, probably due to the presence of acetyl amines derived from muco-proteins. O.R.I.

FOOD VALUE OF DAIRY PRODUCTS

328. Nutritional Effects of the Addition of Meat and Green Vegetable to a Wheat-and-Milk Diet. Experiments with Rats.

H. L. CAMPBELL AND H. C. SHERMAN, Columbia Univ., New York City. J. Nutrit., *16:* 603, 1938.

The complete life cycles were studied in 70 and 69 male and 103 and 105 female rats on two comparable diets, one differing from the other by the addition of fresh meat and green beans to the dry diet. The criteria which were used afforded measures of the diets in relation to growth and development, adult vitality, and length of life.

The addition of fresh meat and green beans was found to result in more rapid early growth, attainment and maintenance of larger adult size, and earlier maturity. It also prolonged very slightly the period of adult vitality as measured by the ability of the females to bear young. It did not significantly influence the length of life. L.A.M.

329. Anomalies in the Bio-Assay of Vitamin D Milk. KENNETH MORGAREIDGE AND BRIAN O'BRIEN, Univ. of Rochester, Rochester, N. Y. J. Nutrit., *16:* 395, 1938.

Experiments are described which show that skimmed milk which has been fortified with vitamin D by the addition of concentrates exhibits, on bio-assay, an enhanced apparent potency which is approximately three times greater than the value expected, based on the amount of concentrate added. Furthermore, it is shown that feeding the concentrate and the skimmed milk separately to the same rats results in less enhancement of potency than does feeding the concentrates in the form of fortified skimmed milk. Milk also enhances the potency of the international standard solution of vitamin D. L.A.M.

330. The Biological Value of Milk and Egg Protein in Young and Mature Rats. EMMA E. SUMNER, Univ. of Rochester, Rochester, N. Y. J. Nutrit., *16:* 129, 1938.

The biological value of whole egg protein is higher than that of whole milk, whether determined by the paired feeding or by the balance sheet method on young or adult rats. Feeding at an 8 per cent level in the diet, the biological value is higher for both egg and milk in young rats than in the year-old animals. In adult rats the biological value for egg and milk proteins is higher at the 5 per cent than at the 8 per cent level of protein in the diet. These facts lead to the conclusion that the protein requirement for growth in young rats and for maintenance in adult rats is not the same. Less protein of good quality is required for the maintenance of mature rats, than for growth in the young. L.A.M.

331. The Availability of Calcium in Spinach, in Skim Milk Powder and in Calcium Oxalate. B. W. FAIRBANKS AND H. H. MITCHELL, Univ. of Illinois, Urbana, Ill. J. Nutrit., *16:* 79, 1938.

In comparison with dried milk the calcium of spinach, in either the fresh, cooked, (open kettle), or canned form, is very poorly utilized for the maintenance and growth of rats. In all probability it is quite unavailable under conditions permitting a very high utilization of milk calcium. Calcium oxalate appears to be entirely unutilizable by the growing rat. L.A.M.

332. Vitamin D and Dental Decay. Milk Dealer, *28:* 6, 62–64, March, 1939.

A review of the literature on the relation of Vitamin D to dental decay. C.J.B.

333. The Calcium Requirements of Pre-School Girls. J. OUTHOUSE, G. KINSMAN, D. SHELDEN, I. TWOMEY, J. SMITH AND H. H. MITCHELL, Univ. of Illinois, Urbana, Ill. J. Nutrit., *17:* 199–211, March, 1939.

Calcium balances were determined on five children during four periods of 4 to 11 weeks each. The maximum average calcium retentions, ranging from 84 to 154 mg. daily, were attained on a calcium intake not greatly in excess to 600 mg. daily by four of the subjects. These subjects received 440 cc. of milk daily. The fifth child apparently needed slightly more, possibly as much as 490 cc. of milk daily. C.F.H.

334. The Lactation-Promoting Effect of l-cystine when Fed with Alfalfa Proteins. L. D. WRIGHT AND J. R. HAAG, Oregon Agr. Exp. Sta., Corvallis, Ore. J. Nutrit., *17:* 263–268, March, 1939.

Alfalfa leaf meal containing 0.37 and 0.18 per cent organic sulphur was fed as 50 per cent of the diet of mother rats. The addition of 0.4 per cent l-cystine to this diet usually increased food intake, approximately doubled the rate of gain of the nursing young, and reduced the body weight of the lactating females approximately 50 per cent.

These results may not apply to practical alfalfa feeding problems, since the level of protein used in this study was only 9 per cent as compared with 12 to 15 per cent level commonly found in alfalfa hay. C.F.H.

335. A Study of Breed and Seasonal Variations in the Ascorbic Acid Content of Certified Milk from Guernseys and Holsteins. A. D. HOLMES, F. TRIPP, Research Labs., E. L. Patch Co., E. A. WOELFFER, H. P. Hood and Sons, Boston, Mass., G. H. SATTERFIELD, Univ. of North Carolina, Raleigh, N. C., J. Nutrit., *17:* 187–198, Feb., 1939.

Six hundred and sixty-one samples of certified milk collected at bimonthly intervals from 55 Guernsey and Holstein cows over a 52 week period indicated a seasonal variation in the ascorbic acid content of milk. The largest amount of ascorbic acid was found in milk produced during February. A second period of high ascorbic acid content occurred in early November.

The ascorbic acid content of Guernsey milk varied from 17.76 to 23.37 mg., and for Holstein milk from 15.72 mg. to 20.44 mg. per liter. The total yearly average for the Guernsey milk was 20.54 mg. of ascorbic acid whereas that of the Holstein milk was 18.17 mg. per liter.

The ascorbic acid content of November certified milk was independent of the ration and period of lactation. The results indicate that the generous consumption of raw certified milk can contribute materially to the vitamin C content of the human dietary. C.F.H.

336. The Biological Value of Milk and Egg Protein in Human Subjects. Emma E. Sumner and J. R. Murlin, Univ. Rochester, Rochester, N. Y. J. Nutrit., *16:* 141, 1938.

The superiority of egg protein over milk protein is less pronounced in the adult human subjects than in young or mature rats. Under the conditions of this experiment, the biological value of milk or egg protein for maintenance in adult human subjects, is lower than observed for maintenance in adult rats, notwithstanding that it was fed to human subjects at a lower level. The proteins of milk and egg are equally well utilized by human subjects or by rats, whether measured by the apparent or true coefficient of digestibility. Egg protein is only slightly superior to milk in replacing nitrogen at the 3 and 4 per cent level in human subjects.

L.A.M.

ICE CREAM

337. Dry Skim Milk in Ice Cream. C. D. Dahle, Dairy Department, Pennsylvania State College, State College, Pa. Ice Cream Field, *33:* 4, 15, 32, 33, 36, 52, 53, 1939.

The author points out the fact that skim milk powder is often used as an ingredient in ice cream. He gives the result of some experiments in which spray powder, vacuum drum powder and atmospheric drum powder are compared one with another and also with condensed skim milk as ingredients of ice cream.

He found a difference of over three minutes in freezing time between mixes using spray process powder and atmospheric roller process powder, and three minutes between the vacuum drum and atmospheric roller process. The scores of the samples five days after making showed that on the average the spray process and vacuum drum process powders gave ice cream of higher quality, but in cases where the powder supplied only a small part of the serum solids the samples containing atmospheric powder sometimes scored as high. When ice cream is held for two months, the difference in scores is not so pronounced, in fact the ice cream made from the condensed milk deteriorated most during such storage. In most cases the ice cream scoring the highest loses the most in score after storage.

The most outstanding difference found due to powders occurred in their physical properties. In mixes containing atmospheric powder the viscosity was greater, the freezing time longer, the acidity higher, the maximum overrun lower and the time required to reach the maximum overrun was also longer. From the point of view of freezing time the spray powder and vacuum drum powder mixes proved very satisfactory and were superior to condensed milk samples which served as the controls.

It is pointed out that these findings are in general agreement with those reported by other investigators. A brief review of some of the results of previously published work is also given. W.C.C.

338. Practical Standardization of Ice Cream Mix with the Aid of the Dairyman's Slide Rule.
B. I. MASUROVSKY. Ice Cream Trade J., *35:* 5, 39, 1939.

Directions are given for the operation of the Dairymen's Slide Rule. With this instrument, it is possible to save time on the solution of milk and cream standardization problems, calculation of ice cream mixes, etc.

W.H.M.

339. Retail Store Accounting and Control.
ROBERT SUTTLE. Ice Cream Trade J., *35:* 5, 66, 1939.

In this the second of a series of articles on retail store accounting, are given an inventory record for supplies other than containers, and directions for keeping this record. An employee's pay roll card is also given, which is designed to give the store manager a complete record of labor cost for a given period of store operation. W.H.M.

340. Sterile Single Service Wins Place in Sanitary Control.
MARY B. ZEVIN. Ice Cream Trade J., *35:* 5, 34, 1939.

Recently there has been put on the market sterile wooden forks and spoons which are wrapped individually, protected for individual use by glassine bags, or by dispensers to assure continued sterility to the point of use. Health departments, sanitary inspectors, proprietors and the public will no doubt appreciate the merits of these sterile, attractive and economical wooden utensils. W.H.M.

341. The Selection and Use of Flavor in Making Chocolate Ice Cream.
W. H. MARTIN, Ice Cream Trade J., *35:* 5, 25, 1939.

This article discusses the various types of chocolate flavor. Types of beans, preparation of chocolate products, composition of chocolate flavoring materials, preparation of chocolate syrup, amounts of flavor to use, preparations of chocolate mixes, freezing chocolate ice cream and how to overcome defects are included in the discussion. Formulae are also given for several special chocolate flavored ice creams. W.H.M.

342. Sherbets. MAL PARKS. Ice Cream Trade J., *35:* 4, 57, 1939.

It is the belief of the author of this article that there is a potential consumer demand for increased consumption of sherbets. The introduction of new sherbet flavors, combination deals which feature sherbet with ice cream, and the more extensive use of sherbets in fountain dishes, were recommended. New uses of sherbets in ice tea, in fountain specials and with meals, have resulted in increased sales. W.H.M.

343. Retail Store Accounting and Control. ANONYMOUS. Ice Cream Trade J., *35:* 4, 30, 1939.

Successful and profitable operation of retail outlets demand informative and timely reports. A retail store report, and directions for its use are presented. The daily report reflects the sales, and inventory of ice cream and sherbet by flavors. From this report the operator can determine the total dollar value of sales and also the value of sales according to different types of sales. The report can be filled out daily by the store manager, and summaries may be made at ten day intervals and at the end of the month. W.H.M.

344. Improvement in Edible Gelatin through Bacteriological Control. R. H. THEXTON, Div. of Bact., Science Service, Dept. of Agr., Ottawa, Canada. Canad. Pub. Health J., *30:* 24, 1939.

In 1935 the Canadian Federal Department of Health passed regulations stipulating that the total bacteria count on food gelatin must not exceed 10,000 per gram with the absence of coli-aerogenes from 1/100 gram. A survey of the bacteriological quality of gelatin imported since these standards were adopted, reveals that gelatin manufacturers have been able to satisfy them in the great majority of cases. O.R.I.

345. Vanilla. R. ROSENBAUM, David Michael & Co. Ice Cream Field, *33:* 4, 10–12, 1939.

The author points out the inadequacy of the present legal standards in protecting the users of vanilla extracts and flavorings. He claims that present methods of chemical analysis are not sensitive enough to detect certain of the flavoring constituents in the small concentrations in which they occur in vanilla flavors. Although the standard tests for vanillin content, "lead number," etc., are made these tests are not sufficient so far as evaluating the flavor. He advocates the use of an educated "taster" as a necessary supplement. It is also claimed that with this organoleptic test in conjunction with regularly used chemical analysis, manufacturers should be allowed to standardize the vanillin content of the vanilla flavors without being forced to label them as synthetic or artificial. W.C.C.

346. Dry Ice. ANONYMOUS. Ice Cream Field, *33:* 3, 9–13, 1939.

A brief review is given of the development of solid carbon dioxide as a refrigerant and many of its applications to the ice cream industry are mentioned.

Refrigerating packages, trucks and dispensing cabinets seem to be the most likely potential uses of "dry ice" in the ice cream industry. Some of the detail problems in each of these fields are discussed. W.C.C.

347. Frosted Foods. LUIS GIBSON, George Gibson Co. Ice Cream Field, *33:* 2, 19–21, 1939.

Several large dairy organizations and a few ice cream manufacturers are already in the frosted food business.

Ice cream is sold mostly during the summer; frozen or frosted foods are sold mostly during the winter. In addition both require storage and delivery at low temperatures. The author states that already the annual production of quick frozen foods is approximately $100,000,000. It is claimed further that the frozen foods business may start by filling in ice cream off seasons, whereas later it may be that ice cream will fill in the off season for quick frozen foods.

State laws have in certain instances prohibited the use of ice cream cabinets for other frozen foods. It is claimed that certain of these laws are in the process of revision so as to permit both these products being stored in the same cabinets.

The frozen foods industry has many problems not common to the ice cream industry. It is necessary to anticipate the demands almost a year ahead. To insure the desired quality correct variety and grade as well as treatment before and during freezing are necessary. In addition proper storage and handling to the time of consumption are imperative. The author suggests that to begin with one should handle a suitable brand of frozen foods rather than try to develop and sell his own brand. Frosted foods are relatively unknown consequently the dealers, retailers and consuming public must be educated to appreciate the merits of such products.

The basic principles of merchandizing must be employed if success is to be expected. Proper display is very important, especially since the products are relatively unknown. W.C.C.

348. Ice Cream Sanitation. C. A. ABELE, Director, Division of Inspection, State of Alabama. Ice Cream Field, *33:* 2, 37–41, 1939.

The author points out that the ice cream industry has made considerable progress in sanitation and its regulation. He claims that a large proportion of ice cream plants now operating in Alabama would have little difficulty in attaining a Grade A rating. The contemplated Grade A ordinance for that state prescribes that an ice cream plant or establishment freezing ice cream elsewhere than in the plant in which the mix is made shall obtain the mix in sealed containers holding just a sufficient quantity for one freezing batch.

Results of bacterial counts on 560 samples of ice cream and sherbet and mix in various stages of manufacture were presented. One set of comparisons are given in the following table.

Comparison of Commercial and Counter Freezer Ice Cream

	No. of samples	Arith. ave. bact. count	Log. ave. bact. count	% of counts under 30,000	% of counts 100,000 or less	% of counts 500,000 or over	% of counts 1,000,000 or over
Commercial Ice Cream	117	154,400	52,000	37.6	65.8	7.8	3.4
Counter Freezer Ice Cream	171	540,200	92,000	32.2	52.1	21.0	15.2

The temperature at which ice cream mix is held in storage was checked on 117 samples. It was found that 3.4 per cent were below 30° F., 23.9 per cent were between 31° and 40° F., 35.9 per cent were between 41° and 50° F. (i.e., 63.2 per cent at 50° F. or below) and 36.8 per cent were above 50° F. with 3.5 per cent above 60° F. The importance of controlling the temperature during transit is emphasized. W.C.C.

349. Lockers Built in Ice Cream Plant. Anonymous. Ice Cream Field, *33:* 4, 16, 17, 1939.

A description is given of a recent installation of cold storage lockers by Johnson Ice Cream Co., West Union, Iowa. The lockers are installed in a room with a flooded ammonia cooling unit manufactured by the King Ventilating System. The cooling unit is centrally located with a blower and ducts diverting the freezing air to four separate rooms. Temperature in the locker room is kept at 12 to 15° F., the quick freezing room at – 20 to – 10° F., the chilling room at 36–40° F. and the fourth room is the ice cream hardening room. The plant has 320 lockers with provision for the installation of 120 more.

Although there are many other cold storage locker systems it is claimed that this is the only one with this particular system of refrigeration.

W.C.C.

350. Use of Stabilizers in Unagitated Ice Creams. Lillian S. Bentley and Betty M. Watts, Dept. of Household Science, Univ. of California, Berkeley, Calif. Food Res., *4:* 1, 101, 1939.

In this study it was found that the stabilizers giving best satisfaction in commercial ice cream are not necessarily the ones producing the best results in unagitated refrigerator ice cream. Agar combined with dry skimmilk produced a coarser texture although considered satisfactory. Pectin gave good results although a flavor effect was noted. Rennet, India gum, egg yolk, cornstarch, etc., were not considered particularly satisfactory. F.J.D.

MILK

351. Recherches sur le Pasteurisation des Laits de Consommation. (Studies on the Pasteurization of Market Milk.) G. Guittonneau, G. Morquot and E. Eyrard. Le Lait, *19:* 113–139, 1939.

Fifty-six strains of the *Escherichia-Aerobacter* group were isolated and submitted to different cultural tests. The A.P.H.A. Brilliant Green 2 per cent Bile medium was found to be a reliable method of detecting all strains. The chief source of *coli* contamination in the raw milk supply was found to be utensils, coolers and cans. In an investigation of high temperature, short time pasteurization, the source of *coli* in raw milk was found to be the result of incomplete sterilization of equipment before use, and recontamination by raw milk, rather than by heat resisting types of organisms surviving this method of pasteurization. O.R.I.

352. The Present Day Challenge to Milk Distribution. Harry L. Gustafson, Secretary, Association of New England Milk Dealers. Milk Dealer, *28:* 6, 84–90, March, 1939.

A discussion is given of the attacks on business which are challenging our present system. The discussion is summarized as follows:

The present day challenge to milk distribution is to tell the facts about the industry and the product it sells.

The challenge comes from employees, producers, consumers, and other distributors.

The facts are available through agencies which the industry has established. Every unit has the responsibility of presenting these facts to the public. It is a challenge which calls for the talents and energies of every man. The time for action has arrived. Let us go forth with the firm conviction that voluntary organization and cooperation under democratic processes in the American fashion is the more permanent and satisfactory way of achieving greater prosperity for all. C.J.B.

353. Sanitation of Paper Milk Containers. J. R. Sanborn and Robert S. Breed, New York State Exp. Sta., Geneva, N. Y. Milk Dealer, *28:* 7, 36–37, 88–90, April, 1939.

A report of a laboratory study of paper milk containers is given. Tables are presented showing the rinse counts obtained from various types of containers as well as the bacterial counts per gram of disintegrated paperboard from which such containers are made. The data show that paper containers can meet much more severe rinse count standards than the average glass bottle, provided the paper containers are properly handled and stored between the time when they are manufactured and the time when they are filled with milk. C.J.B.

MISCELLANEOUS

354. Preserving Cream with Salt. ANONYMOUS. Nat. Butter and Cheese J., *30:* 3, 26, 1939.

O. E. Williams, Bureau of Dairy Industry, U. S. Dept. of Agriculture, Washington, D. C., has invented a method of preserving cream at room temperatures 64° F. dissolving in it about 7 per cent of its weight of sodium chloride. The salted cream can be made practically salt free by mixing it in equal proportions with milk, heating to about 120° F. and separating the mixture to form plastic cream which is then diluted with an equal amount of milk before pasteurizing. The salted cream can be pasteurized and then churned directly into butter. This salt-preserved cream has been used successfully for coffee cream and for making ice cream. Salting lowers the freezing point so that the properties of the cream will not be changed at 0° F. W.V.P.

355. Locker Plant Construction and Design. LE ROY STAUNTON. Celotex Corp., Chicago, Ill. Nat. Butter and Cheese J., *30:* 5, 44, 1939.

Refrigeration engineers have standards for design which give sound construction and prevent avoidable losses. A few are mentioned. W.V.P.

356. Agriculture's Cinderella—The Dairy Goat. F. A. KONIG, Milkdale Farms, Newton, Conn. Milk Dealer, *28:* 6, 80–82, 1939.

A description of the Milkdale goat dairy and a discussion of the goat as related to the dairy industry during the past, present, and future. C.J.B.

357. A Conspiracy Against Business? EDWARD FISHER BROWN, Executive Vice President, Milk Research Council, Inc. Milk Dealer, *28:* 6, 43, 66–70, March, 1939.

The author calls attention to the charges of monopoly and excessive profits to which the dairy industry has been subjected since 1933. To counteract the effect of this propaganda he suggests that the story of the dairy industry since its infancy should be told to the public and that the benefits and protection that the industry has brought to the consumers should be publicized. C.J.B.

358. Value of Medical, Physical Examination of Dairy Employees.

THOMAS A. SCOTT, Pres., Richmond Dairy Co., Richmond, Va. Milk Dealer, *28:* 7, 68-73, April, 1939.

Physical examinations in the milk industry are justified for the following purposes:

1. To select new workers and place them at a type of work for which they are fitted, physically, and mentally.
2. To inform the examining physician with respect to the physical condition of the employee so that he may advise measures for its improvement. Any knowledge gained in this way should be held in strict confidence between the doctor, his assistant, and the individual patient.
3. To inform the employer as to when and where conditions exist in the occupational environment which are apparently causing ill health, or are liable to cause it, that he may take measures for correction or control.
4. To protect those already employed from the risk of communicable diseases, such as tuberculosis and various other infections, which may be introduced into their midst by new applicants.

The author also sets forth several suggestions to follow in instituting plans for periodic examinations. C.J.B.

PHYSIOLOGY

359. The Racial Factor in the Pigeon Crop-sac Method of Bioassay of Prolactin.

ROBERT W. BATES, OSCAR RIDDLE AND ERNEST L. LAHR, Carnegie Institution of Washington, Station for Experimental Evolution, Cold Springs Harbor, New York. Am. J. Physiol., *125:* 4, 722. 1939.

Different races and strains of doves and pigeons may differ markedly in the response (increased weight) of their crop-sacs to prolactin when this harmone is injected either intramuscularly or subcutaneously. When the crop-sac responses obtained are expressed in terms of prolactin units an extreme variation of five-fold was found among six races of pigeons and of eight-fold among fourteen strains of doves. These variations were still more extreme when a high dosage of prolactin was employed. D.L.E.

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

360. Standard Methods for the Examination of Dairy Products. American Public Health Association, 50 West 50th Street, New York, N. Y., and Association of Official Agricultural Chemists, Box 290, Pennsylvania Avenue Station, Washington, D. C. Price $2.50.

This is the seventh edition of the report entitled *Standard Methods for the Examination of Milk,* a publication so well known to the readers of the JOURNAL OF DAIRY SCIENCE that little description is necessary. However, in the present edition methods are included for the examination of ice cream and butter and the title has been changed from one referring to milk and cream only to one including dairy products in general. Other important changes in the seventh edition are; the introduction of methods for the bioassay of vitamin D milk, and improved formula for standard agar, and other changes in the bacteriological section which should help standardize the agar plate technique. Proposed phosphatase methods for the determination of pasteurization are appended. T.S.S.

361. Dairy Science. W. E. PETERSEN. J. B. Lippincott Co. 679 pages, illustrated.

This book, designed primarily as a text for college students majoring in dairy production, presents a rather comprehensive treatment of the field of dairy production and technology. The subject matter is presented in 53 chapters under the following chapter headings.

1. The History of Dairying, 2. Economics of Milk Production, 3. Milk Consumption, 4. Economics of Market Milk, 5. Economics of the Butter Industry, 6. Economics of the Cheese Industry, 7. Economic Phases of the Concentrated Milk Products, 8. Economics of the Ice Cream Industry, 9. Economic Phases of Milk By-products, 10. Origin, Development, and Classification of Dairy Cattle, 11. The Holstein-Friesian, 12. The Jersey, 13. The Guernsey, 14. The Ayrshire, 15. The Brown Swiss, 16. The Milking Shorthorn, 17. The Red Polled, 18. The Lesser Breeds, 19. Types of Dairy Farming and Breed Selection, 20. Selecting the Individual Cow, 21. Fundamental Considerations of Heredity, 22. Measures of the Genetic Makeup, 23. Known Inherited Characteristics in Dairy Cattle, 24. Systems of Breeding, 25. Selection and Care of the Herd Sire, 26. The Purebred Business, 27. Growth and Development, 28. Calf Raising, 29. Raising the Heifer from Weaning to Calving Time, 30. The Cow's Udder—Its Structure and Development, 31. The Physiology of Milk Secretion, 32. Factors Influencing the Quantity and Quality of Milk, 33. Management of Dairy Cows, 34. The Milking Machine, 35. Record Keeping, 36. Efficiency and Cost of Milk Pro-

duction, 37. Common Diseases of Dairy Cows, 38. Dairy Barns, 39. Barnyard Manure, 40. Feeding Dairy Cattle, 41. Common Feeds: Characteristics and Treatment, 42. Feeding Dairy Cows in the Barn, 43. Feeding Cows on Pasture, 44. Chemical Constituents of Milk, 45. Physical and Chemical Properties of Milk, 46. Dairy Microbiology: Bacteria, Yeasts, and Molds, 47. Common Tests of Milk and Cream, 48. The More Common Dairy Processes, 49. Market Milk, 50. Butter, 51. Cheese, 52. Condensed and Dried Milk, 53. Ice Cream and other Dairy Products.

The material in those chapters dealing with specific production problems is dealt with exhaustively while that of those pertaining to other phases of the industry is of a general nature.

This book is well written and generously illustrated. A list of selected references for each chapter and questions covering the material presented in each chapter are helpful additions to the appendix. T.S.S.

362. Dairy Science Abstracts. Imperial Bureau of Dairy Science, Shinfield, Reading, England.

The Imperial Bureau of Dairy Science announces this new abstracting service in the form of Dairy Science Abstracts Vol. 1, No. 1, May 1939. The specimen copy received indicates a comprehensive coverage of the current dairy science literature abstracted under the general headings, *Husbandry, Technology, Control and Standards, Economics, Physiology, Bacteriology and Mycology, Chemistry and Physics, and Miscellaneous.* Published quarterly, annual subscription rate for the United States of America including postage is 25/–. T.S.S.

BACTERIOLOGY

363. Use of Agar Slants in Determining the Sanitary Quality of Milk. G. J. Hucker and W. C. Haynes, New York State Agr. Exp. Sta., Geneva, N. Y. Am. J. Pub. Health, *29:* 6, 651, 1939.

The Burri slant procedure is described which consists in spreading 0.001 cc. of milk with a standardized loop over the dry surface of a 2 per cent agar slant. The method is simple and of practical value for use in controlling the sanitary quality of milk and other products. M.W.Y.

364. The Operation of the Bacteriological Laboratory for Dairy Plants. C. S. Mudge. Calif. Exper. Sta. Circular, *310:* 37, June, 1938.

This circular is designed to supplement the Standard Methods of Milk Analysis by giving such directions as are necessary for the dairy laboratory technician who has not had the advantage of college grade training. Included are a brief description of bacteria, the arrangement of the laboratory, a list of necessary equipment with directions for best results with it, directions for making media, standardization of pH, and instructions for operating the laboratory in all the steps from collecting the sample through plating, incubation, and counting. The manipulations in making the direct microscopic count are described.

Of unusual interest is a section devoted to the proper use of the microscope and a discussion of critical illumination in microscopy. E.L.J.

BREEDING

365. The Relationship of Environment to Selection. L. M. WINTERS, Univ. of Minn. Proc. Am. Soc. Animal Prod., *31:* 278–283. 1938.

From a consideration of the effect of environment on the expression of inheritance it is suggested in proving dairy cattle that the purpose may be defeated under such artificial conditions as prevail in Advanced Registry or show herd performance. G.C.W.

366. Relation of Regression to Selection. W. W. YAPP, Univ. of Ill. Proc. Am. Soc. Animal Prod., *31:* 272–277. 1938.

The prevailing conceptions to account for regression are mentioned, data on the university Holstein herd is presented, and consideration is given to the theory as applied to dairy cattle breeding. G.C.W.

367. Conference on Artificial Insemination.—six papers. Proc. Am. Soc. Animal Prod., *31:* 223–259. 1938.

Includes six papers on recent experiences in the United States and in England on various phases of the subject. G.C.W.

368. Estrus, Ovulation, and Artificial Insemination in Dairy Cattle. G. M. WERNER, L. E. CASIDA, AND I. W. RUPEL, Univ. of Wis. Proc. Am. Soc. Animal Prod., *31:* 54–57. 1938.

Observations were made A.M. and P.M. on evidence of heat in a group of 35 heifers on pasture. Examination for follicles showed none to be ruptured while in heat; 17 to be ruptured by the end of the first day following heat; 19 more ruptured by the end of the second day; and the final 3 between the end of the second and 2½ days following the close of estrum. Reference was also made to experiences with artificial insemination. G.C.W.

369. Nicking in Dairy Cattle. E. E. HEIZER, M. C. HERVEY, G. R. BARRETT, AND G. W. BRANDT, Ohio State Univ. Proc. Am. Soc. Animal Prod., *31:* 67–72. 1938.

To study the theory of nicking, the daughters of sires in a large Ayrshire herd, a large Guernsey herd, and a large Holstein herd were grouped according to the family, or sire relationship, of their dams. Distinct evidence of nicking seemed to be indicated and it was suggested that this is due to complementary effects of genes. G.C.W.

BUTTER

370. Observation on Roll-less Churns. O. F. HUNZIKER, La Grange, Ill. Nat. Butter and Cheese J., *30:* 7, 12, 1939.

The operation of one roll-less churn has been observed since June, 1938. Compared with a roll churn made by the same manufacturer it was found that the roll-less churn shortened the churning period by 6 minutes without decreasing the exhaustiveness of churning; shortened the working period 2 minutes; distributed moisture and salt as uniformly; produced as good body; reduced the yeast and mold count slightly but consistently; worked large and small churnings effectively; and was surprisingly efficient in reworking high moisture churnings. W.V.P.

371. Butter Storage. J. L. PARSONS, W. H. MARTIN, AND D. L. MARTIN, Kansas Agr. Exp. Sta., Manhattan, Kansas. Nat. Butter and Cheese J., *30:* 5, 16, 1939, and 6, 50, 1939.

Storage stocks of butter usually increase in summer and decrease in winter months but the quantity in storage is a small proportion of the annual production. Butter production, consumer demand and the previous year's profit on stored butter affect the volume of butter stored.

Storage charges in Chicago for butter valued at 25 cents per pound equal $0.00359 for the first month and $0.00244 per pound for each succeeding month. Hedging charges and tax increase the cost. Price changes chiefly determine profits and losses in storing butter. Factors affecting butter prices are listed and discussed to show how operators can protect their investment. W.V.P.

372. Application of the Phosphatase Test to Creamery Butter. G. W. SHADWICK, JR., AND M. E. PARKER, Beatrice Creamery Co., Chicago, Ill. Am. J. Pub. Health, *29:* 5, 482, 1939.

A series of experiments was made upon sour cream "flashed" at different temperatures using a laboratory pasteurizer. The results indicate that a temperature of 185° F. or higher will give negative phosphatase reactions both with the cream and the butter freshly made from it. Some indications of possible phenol production by bacterial activity in butter samples subject to the keeping quality test of holding at 70° F. for 8 days were observed. The authors point out that due to factors which may stimulate phenol production in butter during storage, caution should be exercised in interpreting a positive phosphatase test as indicative of the inadequate pasteurization of the cream used in its manufacture. M.W.Y.

373. Studies of Musty Flavor Outbreaks. Harold Macy. Am. Prod. Rev., *87:* 20, 514–515, Mar., 1939.

This article is of preliminary work being done on a frequently reported defect found in some butter samples, known as "mustiness," but more nearly representing a malty flavor. The defect was reproduced by inoculating experimental batches of cream with bacteria closely resembling *Streptococcus lactis var. multigenes.* Suggested control of this type bacteria includes grading out milk and cream showing the flavor, proper care of utensils on the farm, holding cream cold before pasteurization and pasteurization at 160° F. or higher for thirty minutes. P.S.L.

374. Methods of Testing Buttermilk. E. W. Bird. Am. Prod. Rev., *87:* 16, 374–377, Feb., 1939.

The author describes methods in use in testing buttermilk, their limitation, the characteristics of the fatty-like materials found in buttermilk, and calculation of fat losses by formulae. He emphasizes that buttermilk fat test is not so important *per se,* but that fat loss per 100 pounds fat placed in the churn is very important; that the buttermaker's judgment should be regulated by the particular buttermilk test used; and that cream test affects vitally the churning loss. P.S.L.

CHEESE

375. Factors That Influence Cheesemaking. W. S. Arbuckle, Univ. of Missouri, Columbia, Mo. Nat. Butter and Cheese J., *30:* 7, 38, 1939.

A review is given of the characteristic properties and functions of the important constituents of milk in cheese-making. The influence of pasteurization, mastitis and special treatments of milk, curd and cheese on quality of cheese are mentioned briefly. W.V.P.

376. Cut Cottage Cheese Returns 90 Per Cent. Anonymous. Milk Dealer, *28:* 8, 39, May, 1939.

Foremost Dairies, Birmingham, Alabama, have been able to cut down on their returns of cottage cheese from the stores 90 per cent by exposing to ultra rays the cheese, the package, the lid and the surrounding air during the packing process. C.J.B.

CHEMISTRY

377. pH—What It Means. S. T. Coulter, Univ. of Minn., St. Paul, Minn. Nat. Butter and Cheese J., *30:* 6, 18, 1939.

Active acidity or alkalinity is measured by pH while total acidity or alkalinity is measured by titration. The activity of acids (or alkalies) depends on their tendency in solution to dissociate into hydrogen (or hydroxyl) ions. Strong active acids like hydrochloric dissociate completely while weak acids like lactic undergo relatively slight dissociation. Even water dissociates at room temperatures to give $\frac{1}{10,000,000}$ grams of hydrogen ions per liter. For convenience this concentration is converted to pH by the formula:— $\text{pH} = \log 10 \frac{1}{\text{hydrogen ion concentration}}$. The pH of water or true neutrality therefore, is 7.0. A scale shows pH values and hydrogen ion concentrations corresponding to the end point of phenolphthalein titration, iso-electric point of casein, various concentrations of acids and alkalies and acidities of common dairy products. Hydrogen ion concentration rather than titratable acidity influences many chemical changes in dairy products and the actions of enzymes and bacteria. Measurements of pH cannot always be used instead of titratable acidity determinations.

W.V.P.

378. Separation of Lactose and Soluble Proteins of Whey by Alcohol Extraction.

Abraham Leviton and Alan Leighton. Ind. Eng. Chem., *30:* 11, 1305–1311, 1938.

A new process is described for the separation of the constituents of sweet soluble spray-dried whey powder by extraction with 70 per cent or 95 per cent alcohol. The whey powder is first treated with sufficient alcohol to wet it, the paste is then mixed with additional alcohol, and the resulting solution rapidly filtered. The protein, being insoluble in the solvent, is recovered as a precipitate and dried. The fraction contains approximately 50 per cent water-soluble and heat-coagulable whey protein, 15 per cent ash, and 35 per cent lactose. It possesses high whipping properties. After removal of the protein precipitate, the filtrate is treated with HCl to hold in solution any residual protein. Lactose, which was present in the original powder, in an amorphous condition, being supersaturated and insoluble in alcohol, now begins to crystallize. After crystallization is complete, the lactose is separated centrifugally. The alcohol is distilled from the mother liquor, the latter being high in riboflavin content.

When 70 per cent alcohol is used as solvent, 80 per cent of the lactose originally present in the whey is recovered as highly pure alpha lactose. With 95 per cent alcohol as solvent 60 per cent of the lactose in the whey powder in recovered as a very pure mixture of 1.3 parts of β lactose per part of α lactose.

Analytical data concerned with various phases of the process are presented.

B.H.W.

379. A Simplified Quinhydrone Electrode Application in Determining the H-ion Concentration of Liquids and Semiplastic Solids.

George P. Sanders, Bureau of Dairy Industry, Washington, D. C. Ind. Eng. Chem., Analytical Ed., *10:* 5, 275, 1938.

A modified quinhydrone electrode, sample, tube, and calomel half-cell, which were developed chiefly for use on dairy food products, such as milk, whey, and cheese, are described. The electrode is a plain gold-plated platinum wire, and the conventional stopcock in the calomel half-cell is replaced by a sealed-in platinum wire. A simplified sample holder is described. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

380. Spray Drying. Ben B. Fogler and Robert V. Kleinschmidt. Ind. Eng. Chem., *30:* 12, 1372–1384, 1938.

A comprehensive description of the important spray drying systems, and the principles and economics of their operation is presented. The Gray-Jensen unit commonly used in the drying of milk is included. There is a bibliography of the important patents on spray drying. B.H.W.

FEEDS AND FEEDING

381. The Nutritive Value of Alfalfa Proteins as Measured by Lactation in Rats. J. R. Haag and L. D. Wright, Oregon Agr. Exp. Sta. Paper presented at the meeting of the Western Division of the American Dairy Science Association, October, 1938.

The growth of litters of six nursing rats was used as a measure of the ability of rats to lactate on rations containing approximately 9 per cent alfalfa crude protein. The lactation-promoting properties of such rations were enhanced by the addition of 0.4 per cent l-cystine. The average weight of the young produced on the alfalfa ration was 15 grams at 21 days. The average weight of the young produced on the ration supplemented with cystine was 25 grams. The lactating rats lost an average of 90 grams on the alfalfa ration and 50 grams on the supplemented ration. L.D.F.

382. The Utilization of the Nitrogen of Urea and Ammonium Bicarbonate by Growing Calves. E. B. Hart, G. Bohstedt, H. J. Deobald, and M. I. Wegner, Univ. of Wis. Proc. Am. Soc. Animal Prod., *31:* 333–336. 1938.

Quite definite proof is offered that calves on low protein rations make use of urea and ammonium bicarbonate as measured by growth, composition of muscle, and computed storage of nitrogen. G.C.W.

383. The Effects of an Atypical Blindness Producing Ration upon the Vitamin K and C Content of Calf Blood. P. H. Phillips, I. W.

Rupel, J. J. Oleson, and G. Bohstedt, Univ. of Wis. Proc. Am. Soc. Animal Prod., *31:* 320–327, 1938.

On a basal ration of white corn 22 parts, linseed oil meal 23 parts, wheat midlings 11.5 parts, and oat mill feed 40 parts, supplemented with ground limestone, iodized salt, and irradiated yeast atypical A deficiency symptoms developed. The addition of 100 micrograms of carotene per kg. of body weight produced normal growth and health. Without 25 cc. daily doses of Wesson Oil normal blood clotting was lacking, suggesting the need for vitamin K in the calf. G.C.W.

384. **Effect of Decreased Body Reserves of Vitamin E on the Reproduction of Goats.** G. K. Underbjerg, B. H. Thomas, and C. Y. Cannon, Iowa State College. Proc. Am. Soc. Animal Prod., *31:* 63–67, 1938.

As previously reported goats reproduced successfully on vitamin E free rations. Rats fed E depleted diets supplemented with milk, butterfat, muscle or adipose tissue of goats raised on depleted E rations failed in reproduction, whereas rats fed on E depleted diets supplemented with milk and tissues of goats raised on the usual farm ration reproduced successfully. G.C.W.

385. **One Cause of Fat Variation in Milk.** E. B. Powell, Ralston Purina Co. Proc. Am. Soc. Animal Prod., *31:* 40–53, 1938.

The percentage of fat in milk of complete lactations of Holsteins was lowered from 0.5 to over 1.0 per cent by reducing the roughage markedly below the normal amount fed to lactating cows; and when hay was finely cut, or ground and cubed with a grain mixture, there was also a marked decrease in fat percentage. G.C.W.

386. **Determination of Beta-Carotene in Alfalfa Meals.** Richard C. Brooke, Stanley W. Taylor, and Warren S. Baker, Writhmore Research Laboratory, Malden, Mass. Ind. Eng. Chem., Analytical Ed., *11:* 2, 104, 1939.

A method for the determination of beta-carotene in alfalfa products has been developed by modifying the Peterson and Hughes procedure. A special shaker to facilitate the extractions is described, and other steps in the method have been made adaptable to routine analysis. Beta-carotene was determined in the petroleum ether extracts by means of a photelometer. E.H.W.

FOOD VALUE OF DAIRY PRODUCTS

387. **Vitamin D Potency of Human Breast Milk.** Robert S. Harris and John W. M. Bunker, Mass. Inst. of Tech., Cambridge, Mass. Am. J. Pub. Health, *29:* 7, 744, 1939.

A composite sample of 15 gallons of human milk contained only 4 USP XI units of vitamin D per quart. The authors conclude that the antirachitic superiority of human milk over cow's milk in infant feeding cannot be attributed solely to the quantity of vitamin D which it contains. It is possible to increase the antirachitic potency of human milk at least fivefold by feeding metabolized vitamin D milk to the lactating mother.

M.W.Y.

388. Studies on the Metabolism of Calcium and Phosphorus and on the Availability of These Elements from Milk and from an Inorganic Source. K. M. Henry and S. K. Kon, Nat. Inst. of Res. in Dairying, Univ. of Reading, Shinfield, Nr. Reading, England. Biochem J., *33:* 173, 1939.

Considerable effort has been already expended by a number of investigators to ascertain whether pasteurization affects the availability of calcium and phosphorous of milk, and whether calcium and phosphorous from inorganic sources are efficiently utilized as are those salts in milk.

Henry and Kon present a concise referential review of this problem in the light of experiments they had conducted on rats having varying degrees of body storage of the minerals.

In their studies it was observed that the calcium of milk was more available than the calcium from calcium acid phosphate, although under conditions of optimal utilization the difference is small. When excess phosphorus in the form of di-sodium phosphate was used, sub-optimal quantities of calcium were retained from spray dried milk with an efficiency of 98.1 per cent and from mono-calcium acid phosphate with an efficiency of 96.5 per cent, which difference is significant. K.G.W.

389. Vitamins of Elephants Milk. Zofia Markuze. Biochem. J., *33:* 198, 1939.

Elephant's milk has a lower content of vitamin A and D than does cow's milk. Its vitamin B content exceeds that of cow's milk by about twofold. Elephant's milk and cow's milk have about the same content of vitamin B_2 complex. Elephant's milk has about three and one-half fold the ascorbic acid content of cow's milk. K.G.W.

HERD MANAGEMENT

390. Effects of Climatic Factors on Live Stock. F. R. Edwards, Georgia Agr. Exp. Sta. Proc. Am. Soc. Animal Prod., *31:* 48–53. 1938.

Jersey cattle entered in the Register of Merit from Georgia and Maine over a 23 year period from the time if its adoption in 1903 were studied on the basis of the month of birth and butter fat yield. It was concluded that a cow conceived in December and January in Georgia and from October to

May in Maine has a chance of being a better producer than those conceived at other periods of the year. This is attributed to climatic effect during the uterine period. G.C.W.

391. **Raising Dairy Calves in California.** S. W. MEAD. Calif. Agr. Ext. Ser., Circular 107, April, 1938.

Directions are given for caring for young dairy stock divided into the following periods of their lives: from birth to two weeks of age, from two weeks to six months, from six months to one year, and from one year to the first calving.

Other sections include discussion of concentrates and roughages common to California, sanitation, quarters, common ailments together with means of prevention, and the place of vitamins and minerals in the ration. E.L.J.

392. **The Distribution of Elements of Fertility between Feces and Urine in Dairy Cattle.** K. E. HARSHBARGER AND W. B. NEVENS, Univ. of Ill. Proc. Am. Soc. Animal Prod., *31:* 58–61, 1938.

The distribution of N, P and K varied with the character of the ration, amount of food intake, and level of milk yield. The proportion of excreted N in urine varied from 32.3 per cent on a ration of clover hay and timothy hay, silage, and grain with a small amount of protein supplement, to 64.9 per cent on the same ration with a large amount of protein supplement. On the same type of ration the N in urine varied from 51.4 per cent when producing 45 lbs. of milk daily to 60.4 per cent when not lactating. On a ration containing timothy hay K dropped to 22.4 per cent in the urine when normally there is 2 to 3 times as much in urine as in feces. P in urine varied from 0.5 per cent to 6.8 per cent of the total excreted.

G.C.W.

ICE CREAM

393. **Overcoming Seasonal Variations in Ice Cream Sales.** B. I. MASUROVSKY. Ice Cream Trade J., *35:* 6, 18b, 1939.

The author gives statistics showing that of the frozen products manufactured in a year, 24.09 per cent is produced in the spring, 43.90 per cent in the summer, 19.98 per cent in the autumn, and 11.53 per cent in the winter. He believes that sales should be stimulated in the winter so as to give less fluctuation in the quantity produced from one season to the next. In order to accomplish this end a chart is included which gives the special occasions of each month that might be capitalized on for sales stimulation. The leading flavors, new combinations of flavors, and timely fancy forms are given for each month. W.H.M.

394. **Dull Scraper Blades Cost Industry Thousands Every Year.** A. W. FARRALL. Ice Cream Trade J., *35:* 6, 33, 1939.

The author shows how dull scraper blades cut down freezer efficiency, and indicated a saving of 45 per cent in freezing and whipping time after dull blades were sharpened. Sharpening consists of two phases. First is the removal of the burr which must be done rather frequently. The use of a special sharpener is advised. Second is the dressing of the heel of the blade to the proper width, this being necessary at infrequent intervals.
W.H.M.

395. Development and Extent of the Frosted Foods Industry. M. A. SCHAARS, Univ. of Wisc., Madison, Wisc. Ice Cream Rev., *22:* 10, 52, 1939.

Today there are reported to be more than 100 companies freezing food products and using various forms of the four principal quick freezing methods (the immersion, the brine spray, the single plate and the multiple plate). The reported output of all frozen foods in 1937 was 370,933,100 pounds and the estimated output for 1938 is 480,000,000 pounds. In the rearrangement of food distribution, created by the frozen foods industry, the dairy and ice cream distributors stand a chance of playing a very important role. J.H.E.

396. Preserving Cream with Salt. O. E. WILLIAMS, U. S. Dept. Dairy Industry, Washington, D. C. Ice Cream Rev., *22:* 9, 36, 1939.

Cream can be preserved at room temperature without refrigeration for as long as eight days by the addition of 7 per cent sodium chloride. When preparing this cream for ice cream purposes milk is added to the salt cream mixture and the whole is subjected to temperature of approximately 120° F. The cream is then separated from the milk and salt, producing a plastic cream. Fresh milk is added in the proportion sufficient to restore the cream to the required butterfat content. The mixture is then pasteurized and cooled. J.H.E.

397. Creating Consumer Demand for Ice Cream through Advertising. FRANK BEACH, Univ. of Ill., Urbana, Ill. Ice Cream Rev., *22:* 10, 42, 1939.

The critics of advertising are saying that not enough real information is given to enable readers to buy intelligently and wisely. A well-planned advertising program on ice cream should set out to dispel the erroneous opinions many consumers have about ice cream. Other criticisms and also essentials for success in advertising are discussed. J.H.E.

398. Preparation of Sherbets and Ices. W. H. MARTIN, Kan. State College, Manhattan, Kan. Ice Cream Rev., *22:* 10, 38, 1939.

A review of the principles involved in the processing of sherbets and ices. J.H.E.

399. Ice Cream Cabinet Maintenance. B. LEWIS, Meese, Inc., Madison, Ind. Ice Cream Rev., *22:* 9, 31, 1939.

Suggestions are given for getting the best operating efficiency when installing a conversion unit in an old ice cream cabinet. J.H.E.

400. Ice Cream Cabinet Maintenance. R. W. WOODCOCK, Consumers Power Co., Saginaw, Mich. Ice Cream Rev., *22:* 10, 37, 1939.

The advantages of using an electric oven for dehydrating rebuilt and reconditioned sulphur dioxide ice cream cabinets are pointed out. This system enables removal of moisture before the system is charged. J.H.E.

401. Washing and Sterilizing Ice Cream Plant Equipment. H. A. ACKERMAN, Hydrox Corp., Chicago, Ill. Ice Cream Rev., *22:* 9, 52, 1939.

The selection of the proper cleaning agent should be given serious attention. The type of equipment, the material to be removed, and the hardness of the water must be considered. A chemical sterilizer should be selected that is stable in its original form and least corrosive to equipment. In ordinary work use a rinse solution of 100 p.p.m. Tanks should be sprayed with a solution of 300 p.p.m. J.H.E.

402. Some Seasonal and Unusual Types of Ice Cream Specials. D. A. HAASE, Walgreen Drug Stores, Chicago, Ill. Ice Cream Rev., *22:* 9, 41, 1939.

Experiences of a large company in the promotion of week-end ice cream specials are given. J.H.E.

403. Factors Affecting Costs. E. L. SCARAMELLA, Production Manager, Forward Dairy, San Jose, Calif. Ice Cream Field, *33:* 4, 1939.

Costs of manufacture and distribution of ice cream and its related products, sherbet and ice cream are shown. Costs were gathered in several plants over several years. Costs of making 12 per cent mix shown in detail at $0.6002 per gallon with a 40-cent butterfat market. Cost of manufacturing ice cream, shown in detail, show variation on finished cost, due to flavor, of $0.421 to $0.544 per gallon. Costs of manufacturing of ice milk shown as $0.3285 per gallon mix (40¢ lb. fat) and $0.2865 per gallon at $0.309. Affect of using increased serum solids in mix on finished cost of ice cream are shown, and calculations showing how it is brought about are given. The difference is shown to be about 6.1 cents per gallon. Delivery expenses are presented and divided into five classifications showing average costs to be 25 cents per gallon. Comparison of cost of manufacturing and distribution as developed in the article show a margin of 18.1 cents per gallon for ice cream, 34.1 cents per gallon for sherbets, and 6.3 cents per gallon for ice milk. E.L.S.

MILK

404. Use of Phosphatase Test in the Control of Pasteurization. Paul F. Krueger, Board of Health, Chicago, Ill. Am. J. Pub. Health, *29:* 5, 477, 1939.

The phosphatase test, when originally applied, showed a relatively large proportion of samples of milk and cream to be improperly pasteurized, due principally to improper construction and operation of pasteurization equipment. In 88 milk pasteurization plants, 212 outlet valves and 146 inlet valves were defective when compared to the latest standards of the U. S. Public Health Service. Common defects in valves and methods for their correction are discussed in detail. M.W.Y.

405. Sanitary Condition of Paper Containers for Retail Packaging of Perishable Foods. J. R. Sanborn, New York State Agr. Exp. Sta., Geneva, N. Y. Am. J. Pub. Health, *29:* 5, 439, 1939.

This paper is based on bacteriological studies of seven types of paper containers for milk and milk products, and a study of the sanitary condition of the paper board out of which containers are made. During 1938, a marked improvement took place in the bacteriological condition of both paperboard and finished containers over results obtained in 1937. A majority of the containers yielded less than 5 bacterial colonies per container. M.W.Y.

406. The Plant of Tomorrow. V. Schwarzkopf, Vice-President, The Lathrop-Paulson Co. Milk Dealer, *28:* 8, 41, 46–52, May, 1939.

A discussion of the plant of tomorrow from the standpoint of new products, new materials, new methods, new machinery, etc. The following conclusions are drawn by the author:

We can view the future only by what has gone before. Human nature has not changed; human wants are ever greater and greater.

Business in the future as in the past can only survive if it is capable of earning a profit.

Profits can be made but it will require alert, aggressive, progressive management. In the struggle for profits we must not overlook the importance of a carefully planned, well-constructed, properly equipped plant.

In the future as in the past, obsolete methods, processes, plant and equipment will decrease or eliminate profits while improved methods, process and equipment will increase profits.

From 1919 to 1929 American industry spent over 210 billions of capital investment in the form of buildings, tools and equipment—an average of over 19 billions per year—and it produced the greatest period of profits we have ever experienced. Since 1929 the expenditures for capital investment have averaged less than three billion dollars per year. America had been trying to progress without adequate tools and we have had one of the leanest periods we have ever experienced. It is estimated that it would require approximately 65 billions to catch up on our delayed capital investment program, a program to make American industry efficient and capable of meeting present-day requirements.

Each and every one is very much concerned about laws and regulations which restrict or retard business, but these are not our greatest hazard. Such laws and regulations in all probability apply with equal force to your competitors as well as to you. Therefore, you are still on the same competitive basis. But if you have an outmoded, obsolete plant or equipment or have an inefficient, wasteful system that requires excessive upkeep, power and labor, you are at a distinct disadvantage in competition with the up-to-date systems which other plants may have, and your profit margin will be sure to diminish or disappear. Your ability to meet new conditions to keep in tune with the times, to take advantage of new live facts will to a great degree determine your success. C.J.B.

407. Proportional Milk Sampler Patent Held by W. W. Fassett. Milk Dealer, *28:* 111, March, 1939.

In view of a previous article abstracted in this Journal (abstract 21, page A9, vol. 22), this note calls attention to a patent held by W. W. Fassett on a proportional milk sampling device. T.S.S.

MISCELLANEOUS

408. Some Suggestions for Cutting Fuel Costs. C. T. BAKER, Atlanta, Ga. Ice Cream Rev., *22:* 10, 32, 1939.

Fuel wastes can be reduced by preventing excessive heat waste to the stack, providing a stack damper, preventing cold air from entering through cracks in the brickwork and providing suitable grates for the type coal burned. The importance of the recovery of condensate from steam traps, steam radiators, etc., is discussed. J.H.E.

PHYSIOLOGY

409. Effect of Pregnancy Urine Extract on Lactation in the Rat. I. L. HATHAWAY, H. P. DAVIS, R. P. REECE, AND J. W. BARTLETT, Depts. of Dairy Husbandry, Nebr. Agr. Exp. Sta. and N. J. Agr. Exp. Sta. Proc. Soc. Exp. Biol. and Med., *40:* 214, 1939.

Fifty-two lactating rats were employed in studying the influence of a pregnancy urine extract (Antuitrin-S) on lactation. The first injection was made shortly after parturition. The number of young in the litters was fixed at 6 and daily weighings were made. All of the animals were fed the same ration. The injection of 100 r.u. of Antuitrin-S during the first 5 days of the lactation period caused no inhibition of lactation. Larger dosages and dosages over a longer period of time reduced the rate of growth of the young 22 to 33 per cent. R.P.R.

410. Effect of Testosterone on Pituitary and Mammary Gland. R. P. Reece and J. P. Mixner, Dept. of Dairy Husbandry, N. J. Agr. Exp. Sta. Proc. Soc. Exp. Biol. and Med., *40:* 66, 1939.

The daily injection of 200 gamma of testosterone propionate for 15 days into sexually mature spayed rats augmented the lactogen content of their pituitary glands about 40 per cent, caused no change in pituitary weight, induced an extensive development of the lobulealveolar system of the mammary glands, and initiated secretory activity. R.P.R.

411. Initiation of Lactation in the Albino Rat with Lactogen and Adrenal Cortical Hormone. R. P. Reece, Dept. of Dairy Husbandry, N. J. Agr. Exp. Sta. Proc. Soc. Exp. Biol. and Med., *40:* 25, 1939.

Pseudo-pregnancy was induced by injecting 100 r.u. of a gonadotropic principle into each of a group of multiparious rats. The injection was repeated 6 days later. On the 12th day following the induction of pseudopregnancy the animals were ovariectomized and a mammary gland removed. Various hormone preparations were injected subcutaneously every 8 hours until 6 injections had been made. Three or four hours following the last injection the animals were sacrificed and their mammary glands observed macro—and microscopically. Lactation was initiated by injecting lactogen plus the adrenal cortical hormone or lactogen plus the adrenal cortical hormone plus a 20 per cent glucose solution. R.P.R.

412. Effect of Estrogen Injections on Lactogen Content of Female Rat Pituitary. R. P. Reece, Dept. of Dairy Husbandry, N. J. Agr. Exp. Sta. Proc. Soc. Exp. Biol. and Med., *39:* 77, 1938.

The injection of Progynon-B into rats with their ovaries intact augmented the lactogen content of their pituitary glands. The pituitary glands from ovariectomized rats injected with 1000 i.u. Progynon-B daily for 15 days were significantly larger than those from rats with their ovaries intact and similarly treated, but the lactogen content per pituitary was similar.
R.P.R.

413. The Effect of Continued Treatment with Anterior Pituitary Extracts on Milk Volume and Milk-fat Production in the Lactating Cow. S. J. Folley and F. O. Young, Nat. Inst. for Res. in Dairying, Univ. of Reading, and Nat. Inst. of Med. Res., London, N.W. 3. Biochem. J., *33:* 192, 1939.

Repeated injections (12 on alternate days) of prolactin and thyrotropic preparations of fresh ox anterior pituitary lobes into cows in declining lactation results in a pronounced increase in milk yield, the increase being more marked with the prolactin preparations. The activity is evidently not due to the thyrotropic activity of the pituitary extracts, but rather due to prolactin activity (pigeon crop stimulating activity).

The increase in milk yield following injections of prolactin preparation was accompanied by an increase in milk fat content, and nearly 48 per cent fat yield for five successive administrations. With prolonged treatment with prolactin, a plateauing of milk production, and decline was observed.

No significant change was observed in the solids-not-fat content of the milk during the experiments, neither were there any significant variations in the lactose, chloride or phosphatase contents.

The authors conclude that the increase in milk yield and fat content of milk from cows in declining lactation following injection of pituitary extracts cannot be ascribed to a single substance. K.G.W.

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Bushnell, L. D.

Cannon, C. Y.
Carpenter, D. C.
Cave, H. W.
Clevenger, W. L.
Cole, W. C.
Copeland, L.
Coulter, S. T.
Cunningham, O. C.
Cunningham, W. S.

Dahlberg, A. C.
Darnell, A. L.
Doan, F. J.
Dorsey, L. M.
Downs, P. A.

England, C. W.
Erb, J. H.
Espe, D. L.

Fabian, F. W.
Frayer, J. M.
Frazier, W. C.
Fuller, J. M.

Garrett, O. F.
Gelpi, A. J.
Golding, N. S.
Goss, E. F.
Greenbank, G. R.
Gullickson, T. W.
Guthrie, E. S.

Hansen, Arne
Herrington, B. L.
Herzer, F. H.
Holdaway, C. W.
Horrall, B. E.
Huffman, C. F.

Irvin, O. R.

Jacobson, C. O.
Jensen, Chris
Josephson, D. V.

Keith, J. I.
Knight, D.
Krauss, W. E.

LaMaster, J. P.
Leighton, A.
Lindquist, H. G.
Locke, R. B.
Lucas, P. S.
Lush, J. L.

Mack, M. J.
Macy, H.
Mann, A. I.
Marquardt, J. C.
Martin, W. H.
Mead, S. W.
Moore, L. A.
Morris, A. J.
Mueller, W. S.

Nelson, D. H.
Nelson, J. A.

Overman, O. R.

Palmer, C. C.
Parfitt, E. H.
Petersen, W. E.

Price, W. V.
Reid, W. H. E.
Richardson, G. A.
Riddell, W. H.

Schultz, E. N.
Sommer, H. H.
Stark, C. N.
Swope, W. D.

Tarassuk, N. P.
Theophilus, D. R.
Thomsen, L. C.
Thurston, L. M.
Totman, C. C.
Trout, G. M.
Tuckey, S. L.

Webb, B. H.
Weckel, K. G.
White, G. C.
Wilbur, J. W.
Wilster, G.
Wylie, C. E.

Yale, M. W.

JOURNALS

American Creamery and Poultry Produce Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics

Biochemical Journal
Biochemische Zeitschrift

Canadian Dairy and Ice Cream Journal
Canadian Public Health Journal
Certified Milk
Cornell Veterinarian

Dairy Industries
Deutsche Molkerei Zeitung

Endocrinology

Food Industries
Food Manufacture
Food Research

Guernsey Breeders Journal
Ice and Refrigeration
Ice Cream Field
Ice Cream Industry
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Jersey Bulletin
Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Chemical Society
Journal of American Veterinary Medicine Association
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Experimental Medicine
Journal of General Physiology
Journal of Heredity
Journal of Genetics
Journal of Infectious Diseases
Journal of London Chemical Society
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology

Kaeseindustrie
Kolloid-Zeitschrift

Lancet
Le Lait

Milchwirtschaftliche Forschungen
Milchwirtschaftliche Zeitung
Milk Dealer
Milk Industry
Milk Plant Monthly
Molkerei Zeitung

National Butter and Cheese Journal

Pacific Dairy Review
Proceedings of Society of Animal Production
Proceedings of Society of Experimental Biology and Medicine

Scientific Agriculture

Tierernährung
Tierzüchter
Trudy Vologodskogo Molochnogo Institut

Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Zeitschrift für Physikalische Chemie, Abt. A and B
Zeitschrift für Untersuchtung der Lebensmittel
Zeitschrift für Züchtung. Reihe B. Tierzüchtung und Zuchtungbiologie
Zentralblatt für Bacteriologie
Züchtungskunde

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

BOOK REVIEW

414. Bergey's Manual of Determinative Bacteriology, 5th Edition. The Williams & Wilkins Co., Baltimore. Price $10.00.

The only descriptive manual for one interested in the classification of bacteria is Bergey's Manual of Determinative Bacteriology. Five editions of this manual have been published during the past 16 years. The first edition appeared in 1923 and described 832 species of bacteria. It contained 1577 descriptions with reference to the original place of publication. The succeeding editions enlarged upon the classification by including new species and genera of bacteria. This new book is greatly enlarged and contains 1032 pages, which is more than double the number in the first edition. The principal change in this edition, as compared to the previous one, is the increase from 3 to 12 families in the Order of Eubacteriales. The justification for the increase in the number of families lies in the recognition of definitely characterized groups of genera, closely related to each other, yet distinct from the other groups.

Bacteriologists who have tried to systematize our knowledge of bacterial genera and species appreciate the difficulties encountered in preparing a satisfactory classification from material that is inadequate. When one reviews the extensive investigations of pleomorphism, life cycles, variation, dissociation and antigen analysis, it appears that these factors only confuse the classification until these changes have been widely accepted. However, in Bergey's Manual there is evidence of fusing some of this incomplete information in the classification scheme. The manual as a whole shows a marked improvement over the previous editions in spite of some inconsistencies. Time and space do not justify a detailed critical review.

The student and research investigator interested in bacteriologic taxonomy will find the fifth edition of Bergey's Manual of Determinative Bacteriology the most complete collection of references, descriptions and classification of microorganisms that has ever been published. H.H.W.

BACTERIOLOGY

415. Flavor Development in Buttermilk, Cottage Cheese and Sour Cream. B. W. HAMMER, Iowa Agr. Exp. Sta., Ames, Iowa. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 5, 120, Feb., 1939.

Buttermilk, cottage cheese and sour cream ordinarily are prepared with bacterial cultures of the same general nature. In a ripening culture roughly 15 to 28 per cent of the lactose is changed to lactic acid by the common lactic acid organism *Streptococcus lactis.* This compound has an acid taste but is odorless and does not influence the aroma. The citric acid in milk is fermented by *Streptococcus citrovorus* and *Streptococcus paracitrovorus.* Some carbondioxide is formed which influences the flavor, and acetic acid which influences both flavor and aroma. In rather acid milk some citric acid goes to form acetylmethylcarbonol and diacetyl, the latter having a pleasing flavor effect on various products. The addition of .15 per cent of citric acid to the milk to be made into culture definitely affects the flavor of the ripened product. The acid may be dissolved in a small amount of water with heat and added slowly with considerable agitation at time of inoculation. Commonly, an increase in carbondioxide, acetic acid and diacetyl contents results. Disappearance of diacetyl by reduction is best controlled by low holding temperatures after suitable ripening has been attained.

Lack of flavor in buttermilk may be due to inactivity of citric acid fermenting organisms, inadequate supply of acid, rapid destruction of diacetyl or some other factor. The best way of preserving the desirable flavor of buttermilk is by cooling and holding the ripened product at a low temperature.

Cottage cheese may be low in flavor due to the flavoring materials being washed out and due to destruction of organisms by high cooking temperatures. Such cheese usually keeps well. High flavored cottage cheese suggesting the flavor of fine culture is commonly preferred by consumers who are critical of the quality of their dairy products. This product requires more care in manufacture than the low flavored product and should be held at satisfactory temperatures if the quality is to be maintained. Attempts are constantly being made to isolate cultures which will be more suitable for flavor development than those now commonly used. E.F.G.

DISEASE

416. Report of Committee of Communicable Diseases Affecting Man.

I. A. Merchant, Iowa State College, Ames, Iowa. J. of Milk Techn., *1:* 26–35, Sept., 1938.

A table showing the various milk-borne epidemics reported in the United States and Canada for 1936 together with their origin is given.

The importance of the phosphatase test for pasteurization as an epidemiological aid is cited. In this case an outbreak of about 75 cases of gastroenteritis was found to be due to a can of pasteurized milk which the phosphatase test proved to be raw.

Brucellosis (undulant fever) still holds a prominent place. In 1936 a total of 2044 cases were reported. These cannot all be attributed to milk, but nevertheless many cases are. That steps are being taken to rapidly remove this hazard is shown by the fact that during the fiscal year 1936, the agglutination test for Bang's disease was made on 6,600,000 cattle (a considerable number of these, however, were retests) and approximately 445,000 reactors were found.

It is suggested that poliomyelitis cases should be thoroughly investigated to determine their contact with milk supplies. L.H.B.

417. A Study of a Scarlet Fever Epidemic at Rockford, Illinois.

C. W. ANDERSON, Director of Laboratories, Rockford Dept. of Public Health, AND S. V. LAYSON, Milk Sanitarian, Illinois Dept. of Public Health. J. of Milk Techn., *1:* 15–19, Sept., 1938.

A total of 48 cases of scarlet fever occurred from March 23 to April 9, 1937. Forty-one of these cases were users of raw milk from one station. Evidence seems to indicate that one cow in this herd had become infected from a milker.

It is interesting to note that Rockford has a compulsory pasteurization law, but jurisdiction does not extend beyond the city limits. Cash and carry raw milk stations have sprung up just outside the city limits. It was to one of these stations that this epidemic was traced. L.H.B.

418. Studies on Milk Samples from Bang-Positive and Bang-Negative Cows.

H. B. MORRISON, JR., Dairy Section, AND F. E. HULL, Dept. of Animal Pathology, Univ. of Ky. J. of Milk Techn., *1:* 3–8, July, 1938.

The results of a study made on four herds with reference to the incidence of mastitis in Bang's disease reactors as compared to non-reactors are given.

Milk samples were taken aseptically from each quarter of each cow's udder. The samples were tested to determine the agglutination reaction to *Brucella abortus,* the presence of streptococci, the brom thymol blue reaction, and the number of leucocytes per milliliter.

From the results obtained the authors came to the following conclusions. A high percentage of Bang's disease reactors gave milk with an agglutinin titer for *Brucela abortus* of 1:50 or higher. A higher percentage of Bang's disease positive cows gave positive reactions to tests for mastitis than non-reactors. Each of these diseases in itself causes severe economic loss to the dairyman. This large economic loss may make it easier for the milk sanitarian to secure the cooperation of the dairyman in efforts to eliminate them. L.H.B.

419. Some Results of Calfhood Vaccination Against Bang's Disease.

J. G. HARDENBERGH AND A. M. MILLS, Walker-Gordon Laboratory Co., Inc., Plainsboro, N. J. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 6, 139, Feb., 1939.

As an aid in the control of Bang's disease economically in herds until such time as the eradication program can be feasibly undertaken the calfhood vaccination method, as worked out by Cotton, was studied. Numerous groups of heifer calves from 4 to 8 months of age have been inoculated with Strain 19 vaccine. The local swellings and elevations of temperature were transient. Agglutination reactions in the vaccinated heifers usually disappeared in 4 to 6 months. Although abortions were observed in both vaccinated and control animals the abortions which could be ascribed to Brucella infection alone occurred to a lesser extent in the vaccinated animals than the controls. E.F.G.

FEEDS AND FEEDING

420. The Effect of Feeding Vitamin A Supplement to Dairy Cattle.

J. G. ARCHIBALD AND C. H. PARSONS. Mass. Agr. Exp. Sta. Bull. 357, March, 1939.

The effect on growth, reproduction, and milk production in dairy cattle, of adding Vitamin A supplement in concentrated form to the grain ration was studied during a period of twenty-two months. All the dairy animals in the State College herd except young calves and bulls were included in the feeding trials.

The supplement used was a fortified cod liver oil.

The results obtained showed that supplement was without effect on the growth of heifers past the calfhood stage.

It had a slight, but not significant, favorable effect on reproductive function in cows and heifers, chiefly reflected in a higher weight at birth of the calves born to the group of cows receiving the supplement and in a smaller percentage of stillborn calves in that group. All phases of reproductive function considered, the trend, although slight, was in favor of the supplement.

The effect on milk production was favorable and seemed to have been brought about through an increased persistency in milk flow by those cows receiving the supplement.

The average butterfat content of the milk was not affected, either favorably or adversely.

The vitamin A content of composite milk samples from cows receiving the supplement was somewhat higher than that of similar samples from cows that did not receive it. The efficiency of transfer of the vitamin from feed to milk was very low in all cases.

The authors considered the slight increase in cost of grain mixtures fortified with a vitamin A concentrate a cheap insurance against troubles due to deficiency of this vitamin particularly when roughage is of poor quality. H.G.L.

421. Haying in the Rain, A Study of Grass Silage. J. G. ARCHIBALD AND C. H. PARSONS. Mass. Agr. Exp. Sta. Bull. 362, April, 1939.

A discussion of different factors to consider in making grass silage are given. The authors suggest that any of the annual or perennial grasses or legumes, or the small grains can be successfully ensiled provided they are cut at the proper stage of maturity. A moisture content of 60 to 75 per cent is recommended when plants are put in the silo. Molasses is recommended at a rate of 40 pounds per ton of green weight for grasses and small grains, 60 pounds for mixed grass and clover, and 75–80 pounds for clover, alfalfa and other legumes. Silage from either grasses or legumes is usually higher in proteins, fiber and minerals and lower in carbohydrates than corn silage.

Feeding practice is similar to corn silage, but large amounts of legume silage should not be fed at first to cows unaccustomed to it. In palatability, legume silage was equal to corn silage. Grass silage was not as readily eaten but was cleaned up without waste. The young cattle seem to relish it as well as they do corn silage. When dry hay is fed along with grass silage, cows seem to clean up their allowance if the silage is fed at one time of day and the hay at another. It probably cannot be fed to the entire exclusion of dry hay.

Feeding trials showed it to be equal to corn silage and superior to dry hay for milk production. Fat test was not significantly different on grass silage and corn silage but there was more difference in favor of the grass silage when it was compared to dry hay. Vitamin A content of the milk was considerably higher when cows were fed grass or legume silage than when they received dry hay, and flavor of the milk was also superior.

Limited cost data indicate there will be little difference in producing grass silage and dry hay on per unit dry matter basis but will vary depending on the season. Grass silage will be generally less expensive than corn silage.

Quality of grass silage depends on the condition of fodder stored for it will not make good feed out of poor or over-ripe hays. It will find a definite place in the farming system but it is doubtful whether it should replace ordinary hay making entirely. H.G.L.

422. Limited-Grain Feeding and All-Year Pasture for Dairy Cows. C. E. WYLIE AND L. R. NEEL. Tenn. Agr. Exper. Sta. Bull. 163, Jan., 1938.

Two groups of dairy cattle were fed two different rations in order to determine the value of limited-grain feeding and the use of good pasture. One pound of concentrates was fed to six pounds of milk in comparing with a check ration of one pound of concentrates to three pounds of milk. The milk and butterfat production on a limited grain ration was about equal to the full grain ration and was slightly in favor of the former in the income over cost of feed. C.E.W.

FOOD VALUE OF DAIRY PRODUCTS

423. New Irradiator Puts 400 Vitamin D Units into Quart of Milk.

ANONYMOUS. Milk Dealer, *28:* 9, 32–33, June, 1939.

A brief description is given of a new process for irradiating milk which has been tested over a period of two years in actual commercial use at Devine's Strawberry Hill Dairy, Norwalk, Connecticut. The new process raises the former limits of 135 U.S.P. units of Vitamin D per quart of milk to 480–500 units per quart without imparting any sign of off-flavor to the milk as the result of ultra-violet light exposure. This equipment has produced milk containing as high as 1,600 units without the flavor of the product being affected in any way from over-exposure to ultra-violet light.

C.J.B.

424. Color and Vitamins in Winter Milk from High Quality Hay and Silage.

G. BOHSTEDT, Univ. of Wis., Madison, Wis. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 6, 148, Feb., 1939.

Most roughages, especially when bleached, heated or harvested when fully matured, are low in carotene and vitamins. In vitamin content grass and legume silages, especially when cut at an early stage and properly preserved, come nearest to pasture. The A.I.V. process, by reducing fermentation in the silo, aids in the preservation of the carotene. The carotene and Vitamin A content has been increased 60 per cent and 125 per cent respectively by an A.I.V. ration compared with corn silage. Although total carotene plus Vitamin A increases were similar, per unit of fat for Guernseys and Holsteins, the latter breed changed a larger proportion of the yellow carotene to colorless Vitamin A and, therefore, gave a whiter milk when the same Vitamin A activity was present per pound of fat as in the Guernsey milk. The use of 65 pounds of molasses per ton of silage is recommended for alfalfa. Only when the original silage material is in the early leafy succulent stage and when the acid method or whatever other method will preserve the material is used, can high color and vitamin content be obtained in winter milk. So much depends upon the condition of the original silage material and the fermentation in the silo that often a high grade of corn silage will be equal to the product of some of the other supposedly superior silage processes. Artificial drying of early cut hay is an effective way to improve the quality of winter milk. E.F.G.

425. The Cow—Mankind's Foster Mother.

W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 6, 143, Feb., 1939.

The effect of the feed of the animal upon the food value of milk, particularly the content of the various vitamins, is discussed. The statement is made that feeding practices which tend to improve the nutritive value of milk are in keeping with good farm practice. The suggestion is made that possibly an evaluation of the feeding program in operation on a farm might be included in the sanitary inspection of the farm. E.F.G.

ICE CREAM

426. Fruit Products in Ice Cream and Sherbets.

D. G. SORBER, Associate Chemist, Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Los Angeles, Calif. Ice Cream Field, *33:* 5, 22–25, May, 1939.

According to the author, fresh fruits possess the most delicious flavors known, yet other flavors are used more extensively as flavors for ice cream. Despite this it is claimed that ice cream is the second largest user of barrel-pack frozen strawberries.

The author recommends more general use of frozen fresh fruit pulp or puree, and frozen crushed or chopped fruit claiming that these products will result in the production of a flavor base wherein the natural fresh fruit flavor and color are fully preserved. Furthermore, such products can easily be prepared. The preparation consists of reducing the fruit to a pulp or puree, adding a predetermined amount of sugar or syrup with proper mixing, packing in tight containers and freezing rapidly enough to prevent deterioration by enzyme activity or the growth of microorganisms. Various types of containers may be used. If tin cans are used they should be enamel-lined or lacquered in order to preserve the natural color of many fruits. Otherwise, the anthocyanin pigment in many fruits such as berries, plums, etc., combine with the tin salts formed by the fruit acids resulting in an undesirable blue color.

Material of the type described can be defrosted and used to advantage as a flavor base for ice cream, sherbets, and milk shakes. When frozen very rapidly it produces a delicious frozen dessert possessing the natural flavor and color of the fresh fruit with a pleasing smooth texture. Crushed and chopped fruits prepared in this manner can be defrosted and used as very satisfactory sundae toppings. W.C.C.

427. Ice Cream Sanitation and Hygiene.

C. A. ABELE, Director, Division of Sanitation, State Dept. of Health, Montgomery, Ala. J. of Milk Techn., *1:* 23–25, July, 1938.

A report of results on a study made of bacterial counts on ice cream and ice cream mix during 1936 and 1937.

Bacterial counts have shown an improvement in 1937 over 1936 in all phases of the study. Counter freezer ice cream showed a higher count than did plant manufactured ice cream, although there was a decided improvement in 1937 over 1936 in the counts of counter freezer ice cream.

Bacterial counts of ice cream at soda fountains were higher than of the ice cream at the plants, indicating possible contamination from dippers as a factor.

The bacterial counts of ice cream were generally higher than were the counts on the mix from which they were made.

Every ice cream freezing plant in Alabama is now required to obtain a permit from the health officers, and every prospective freezer operator must first obtain a permit before starting operation. L.H.B.

428. Flavors in Use. C. D. Dahle, Ice Cream Field, *33:* 6, 36–40, June, 1939.

The author gives a general discussion of the more commonly used ice cream flavors.

He advocates the use of pure vanilla and points out that with chocolate ice cream the tendency is to a blend of chocolate and cocoa. The larger ice cream manufacturers use frozen strawberries in making strawberry ice cream. For small manufacturers he claims processed berries in number 10 tins are satisfactory.

With butter pecan ice cream salt is essential to the development of the best flavor. The use of nuts packed in syrup has the advantage of delaying the development of sandiness in ice cream. Fruit emulsions, it is claimed, developed an unpleasant flavor as a result of oxidation if held too long.

W.C.C.

429. Selling Frosted Foods. E. W. Rosenheim, United Frosted Foods, Inc. Ice Cream Field, *34:* 1, 32, 33, July, 1939.

The author states that first of all frosted foods must be bought right. This means that:

(a) the quality must be uniform,
(b) the packages must be uniform,
(c) the varieties frozen must be correct,
(d) there must be a proper cabinet plan, and
(e) a suitable advertising plan.

In the second place he points out that frosted foods must be sold right. Under this heading he recommends (a) refrigerating the products at about 0° F., (b) using properly refrigerated delivery trucks, and (c) serving the dealer at regular intervals to ensure an adequate supply of the various products in the dealer's cabinet. He points out that this last step is now being used to advantage in other industries and estimates that 95 per cent of the cheese and mayonnaise business is handled through this type of truck distribution. W.C.C.

430. Frozen Cream Institute Survey Analyzed. C. D. Dahle and D. V. Josephson. Ice Cream Field, *34:* 1, 21, 22, July, 1939.

This is a summarized report on the Institute Survey on Frozen Cream. According to the report the "composite opinion" may be outlined as follows:

1. Select good flavored, low acid 40 to 50 per cent cream.
2. Pasteurize at 160° F. or above.
3. Store in tin cans at a temperature of approximately $-10°$ F.
4. Remove the cream from storage and incorporate into the mix using it as a source of 30 to 50 per cent of the total fat of the mix.

It is pointed out that this "composite opinion" is in accord with experimental results obtained at the Pennsylvania Agricultural Experiment Station. The following compilation gives the recommendations of the authors.

1. Select fresh, good flavored, low acid cream containing 40 to 50 per cent fat.
2. Process it in equipment that is as free as possible from exposed copper or even copper-containing alloys.
3. Pasteurize the cream at 170° F. or higher for at least 15 minutes.
4. If sugar is desired, approximately 10 per cent should be added to the cream prior to pasteurization.
5. Store cream in well tinned cans at a temperature of $-10°$ F. or lower.
6. If possible, remove the cream from storage and use within six months after storage.
7. Incorporate the product into the mix so that 30–50 per cent of the fat will come from this source. Larger percentages may be used if the cream is of exceptional quality.
8. Use a small quantity of egg yolk in the mix if difficulty is experienced in obtaining overrun with batch freezers. W.C.C.

MILK

431. An Evaluation of the Various Procedures for Making Phosphatase Tests. L. H. Burgwald and E. M. Giberson, Dept. of Dairy Techn., Ohio State Univ., Columbus, Ohio. J. of Milk Techn., *1:* 11–24, Nov., 1938.

All of the laboratory tests are of approximately equal value in detecting irregularities in the pasteurization of milk. As little as 1° F. variation in temperature, 5 minutes' variation in holding time and 0.1 per cent added raw milk to properly pasteurized is detectable. Scharer's standard of 0.5 p.p.m. of phenol as an indication of proper pasteurization of 143° F. for 30 minutes seemed to be a little low. His test, however, has the advantage over Kay and Graham's and Gilcreas and Davis' in that it requires a much shorter incubation time, and is less expensive in that acid washed filter papers are not required.

Scharer's improved rapid field test is nearly as effective as are any of the laboratory tests and has the distinct advantage of time and cost of operation in its favor, together with the fact that it can be made directly in the plant in a few minutes' time.

BQC was found to vary in sensitivity, and might be a factor in preventing the Scharer tests from being as sensitive as either the Kay and Graham or Gilcreas and Davis tests.

A photelometer can be used in connection with any of the laboratory tests.

Age of samples if kept cold so they do not sour has no effect on the test. Certain bacteria may be a factor with the Kay and Graham and Gilcreas and Davis tests if present in large numbers. This is rather remote, however, and the use of a control test would indicate the presence of any phenol-like substance which they might produce. L.H.B.

432. **Metals Used in a Modern Dairy.** LOOMIS BURRELL, Cherry-Burrell Corp., Chicago, Ill. J. of Milk Techn., *1:* 25–28, Nov., 1938.

The various metals used in the manufacture of equipment are discussed giving their advantages and disadvantages and the equipment they are best suited for.

The trend toward future equipment and processes are also discussed briefly. Among these mentioned is the trend toward high temperature short-time pasteurization, homogenized milk, and general improvements in design of equipment for sanitary reasons. L.H.B.

433. **Report of Committee on Milk Plant Practices.** A. R. TOLLAND, Chairman, Health Dept., Boston, Mass. J. of Milk Techn., *1:* 38–43, Sept., 1938.

The value of proper pasteurization of milk as a health measure is shown in this report for the city of Boston. In 1907 when pasteurization by the holding method was first set up in Boston, there were 760 deaths of children under two years of age due to diarrhea and gastroenteritis. In 1935 with 99.8 per cent of the milk supply pasteurized there were only 49 such deaths.

Higher temperatures and lower caustic content in soaker washers seems to be the trend. Heat penetrates quicker than caustic.

The proper handling of cream is discussed quite fully. A set-up for handling cream to maintain the normal viscosity, they found to be as follows:

Separate milk at 85° F. using an air-tight separator. Run cream into a small stainless steel spray vat. Pasteurize the cream at 145° F. for 30 minutes. Heat the cream slowly using a 15° F. differential between the cream and heating medium at all times. Cool slowly in the vat to 40° F. with ice water.

Plant design and arrangement is discussed as are wet surface paints and various methods of waste disposal at country plants. L.H.B.

434. Problems Related to Homogenized Milk. F. J. DOAN, Dairy Dept., Pennsylvania State College, State College, Pa. J. of Milk Techn., *1:* 20–25, Sept., 1938.

Piston, rotary and sonic homogenizers, and the effect of homogenization on the milk is discussed.

The most notable effects of homogenization are on the fat globule size, tension of the curd, and on the development of oxidized flavor.

Comparison of piston and rotary type homogenizers is given, with reference to effect on curd tension and fat globule size. Rotary types give better results than piston types at 1,000 pounds pressure, but piston types at 3,000 pounds' pressure show much better results than rotary. L.H.B.

435. A Glass Milk Bottle with Narrow Pouring Lip and Minimum Drip. LLOYD ARNOLD, M.D., Univ. of Ill., College of Medicine, Dept. of Bacteriology and Public Health, Chicago, Ill. J. of Milk Techn. *1:* 5–14, Sept., 1938.

A standard pouring machine and technique for determining the amount of drip and bacterial contamination when milk is poured from glass bottles is described. Experiments with various types of bottle finishes and pouring lip designs have been made, and are illustrated.

By breaking the adhesive surface of the glass with recessed angles the amount of drip both during and after pouring milk from the bottle has been reduced to a minimum. L.H.B.

436. "Hit or Miss" Methods of Controlling Washing and Sterilization. F. M. SCALES, Research Laboratories, Sheffield Farms Co., New York City. J. of Milk Techn., *1:* 39–47, July, 1938.

"Hit or miss" cleaning operations are defined, "as those which have not been carefully planned and executed."

A planned operation should consider:

1. Hardness of water to be used.
2. Equipment material to be cleaned.
3. The part of the process for which the equipment is used.
4. The powder that is proposed to be used.
5. The temperature of the cleaning solution.

Desirable detergent qualities are listed as follows: wetting, foaming, deflocculating, solubility, emulsifying and lubricating.

The value of sulphated alcohol in combination with detergents for softening water and increasing germicidal action is shown.

Two methods are proposed for more uniform control of detergent strength.

Methods of sterilization of equipment is discussed. Chlorine solutions are gaining in favor. L.H.B.

437. The Microscope in the Production of High Quality Milk. C. S. Bryan, Mich. Agr. Exp. Sta., East Lansing, Mich.; G. J. Turney, W. K. Fox, L. H. Begeman, and X. A. Miles, Health Dept., Lansing, Mich.; and J. S. Bryan, Castanea Dairies, Trenton, N. J. J. of Milk Techn., *1:* 26–34, July, 1938.

A score card for determining the quality of a patron's milk has been devised, using direct microscopic count, sediment test, and temperature of the milk in determining the score. A passing score is 75.

A key based on the morphology of microorganisms is given to aid in the determination of the possible source or cause of high bacteria counts when found.

The major sources or causes of high bacterial counts are given for four classes of milk. L.H.B.

438. Improvements in the Rapid Phosphatase Test for Detection of Improper Pasteurization of Milk and Its Products. H. Scharer, Chemical Laboratory, Dept. of Health, New York City. J. of Milk Techn., *1:* 35–38, July, 1938.

Directions for conducting the proved rapid field test are given in detail, together with instructions for making up permanent color standards for use with the test.

This test, due to the improvements in the purity and stability of chemicals needed, and the use of (neutral) normal butyl alcohol to extract the indophenol formed by the test, have resulted in yielding a rapid test which is comparable in accuracy to any of the longer laboratory tests. L.H.B.

439. Second Conference on Sanitation of Paper Milk Containers. R. S. Breed, Chairman, New York Agr. Exp. Sta., Geneva, N. Y. J. of Milk Techn., *1:* 47–53, July, 1938.

A report of this conference is given together with a supplementary note on sanitary inspection and control at pulp and paper mills.

The principles of sanitation to be observed in the manufacture and use of paper containers for milk as revised at the conference held at Geneva, N. Y. May 2, 1938, are listed. L.H.B.

440. Contamination of Pasteurized Milk by Improper Relative Pressures in Regenerators. A. W. Fuchs, Senior Sanitary Engineer, United States Public Health Service. J. of Milk Techn., *1:* 9–16, July, 1938.

Milk regenerators may permit the contamination of the pasteurized product unless the pasteurized milk or the heat transfer water are under greater pressure at all times than is the raw milk. Objective criteria are presented, and methods for automatically insuring the required relative pressures in various types of milk to milk and milk to water to milk regenerators are described. L.H.B.

441. **Physical Factors that Influence the Sale of Milk.** W. H. E. REID, Dairy Department, Univ. of Mo. Milk Dealer, *28:* 9, 56–62, June, 1939.

The following physical factors are discussed in relation to their influence on the sale of milk: Effect of pasteurizing temperatures on the cream column; effect of freezing on appearance and flavor; relation of creaming ability to cooling; a bluish skim milk layer; flaky milk; mottled or curdy cream line; and coagulation of milk during cooking in the home.

C.J.B.

442. **Average Retail Price of Regular Milk Is 11.8 Cents, Survey Shows.** ANONYMOUS. Milk Dealer, *28:* 9, 42–46, June, 1939.

A compilation recently made by the Milk Dealer from price reports gathered in 42 states shows that the average retail price per quart of regular milk in the United States today is approximately 11.8 cents. Prices in the South, with an average of 13.4 cents per quart, are higher than in other sections of the country. Eastern prices are second highest, with an average of 12.4 cents per quart; Western prices third, with an average of 11.4 cents per quart; and midwestern prices lowest, with an average of 10.7 cents per quart. Pints of milk for the United States as a whole sell at an average price of 7 cents.

Grade A milk prices for the country as a whole average almost one cent a quart higher than regular milk—12.7 compared with 11.8 cents for regular milk. Vitamin D milk averaged one-half cent above Grade A milk or 13.2 cents a quart. Certified milk averaged 16.8 cents, while certified vitamin D milk averaged 17.5 cents a quart.

Other average prices for the United States as a whole are as follows:

Buttermilk, 8.9 cents a quart; chocolate milk, 12.6 cents a quart; heavy cream, 20.4 cents per half pint; medium cream, 15.4 cents per half pint; light cream, 21.9 cents per pint; sour cream, 12.8 cents per half pint; cream cottage cheese, 14.7 cents per pound; and plain cottage cheese, 12.7 cents per pound. C.J.B.

443. **Reducing Icing Costs.** ARTHUR ADAMS, FlakIce Corporation of New York, AND R. E. MILLER, York Ice Machinery Corp. Milk Dealer, *28:* 9, 36–37, 64–66, June, 1939.

A description of the FlakIce installation, consisting of six 10-ton FlakIce machines, two double-ended storage bins equipped with reversible Flak-

Ice automatic rakes and one FlakIce automatic icer, in the 57th Street plant of Sheffield Farms Company in New York City.

Advantages which the Sheffield Farms Company has obtained through its FlakIce installation include the following:

Ice requirements for crate icing and top icing of trucks can be met at much lower cost than by any other method.

Elimination of labor which would be needed for manufacturing and handling the requirements with block ice.

Elimination of ice wastage which is incurred in the manufacture, crushing and handling of other forms of ice.

A saving of one-quarter to one-third of former block ice requirements in the form of crushed ice for the same duty.

Elimination of un-iced and under-iced crates and elimination of wastage of ice by having too much ice on some crates.

The saving of the cost of a crew of several men who would be required to handle and shovel ice onto milk bottle crates. C.J.B.

444. When Do We Graduate? Michael J. Riordan, Sales Manager, Marin Dairymen's Milk Co., Ltd., San Francisco, Calif. Milk Dealer, *28:* 9, 35, 68, June, 1939.

It is pointed out that the per-capita consumption of milk is, generally speaking, about one-half pint per day. In other words, practically all milk is sold for consumption by infants and growing children, and this certainly requires no salesmanship of even elementary grade caliber because the health authorities see to it that the children's food rations contain large quantities of milk.

Methods of entering the graduating class of milk salesmanship by invading the field of adult milk consumption are briefly discussed by the author. He concludes that: If we of the milk industry would effectively demonstrate to the people of America the myriad uses of fresh milk, the volume of milk sales would increase at least 50 per cent. C.J.B.

445. Unit Coolers for Milk Storage Rooms. C. T. Baker, Consulting Engineer, Atlanta, Ga. Milk Dealer, *28:* 9, 30–31, 70, June, 1939.

A discussion of the advantages of unit coolers over standard type coils for cooling milk-storage rooms.

The author summarizes the advantages of unit coolers when applied to milk-room cooling as follows: They provide better, more uniform and more rapid cooling of the air and the room contents than may be obtained by other methods; they maintain dry conditions, thus eliminating moisture deposits on walls, floors and ceiling; improved sanitation results; they eliminate fog; they protect crates against rust; and increase room storage space. C.J.B.

446. How We Have Used the Films and the Results Secured. FRANK F. RENNIE, JR., Virginia Dairy Company, Inc., Richmond, Va. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 7, 159, March, 1939.

A precise and detailed outline is given of the way in which the Sound Slide Films of the Association were used by a 48 route dairy to train route salesmen. Included were methods of meeting the problem, of when and where to hold meetings, how to get full attendance and organization of the meeting. Participation by the salesmen themselves through discussion, personal experiences and specific sales situations are stressed. Use of the sound films in this manner showed a 1 per cent increase in sales in August, 1938, when 136 leading markets showed a 5 per cent decrease for the same period. E.F.G.

447. A Systematic Method of Reducing Customer Turnover. ROBERT A. BURNS, Whiting Milk Company, Boston, Mass. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 7, 159, March, 1939.

Recent reductions in milk sold are attributed to (1) increased use of soft drinks, (2) increased sales of evaporated milk, (3) the effect that 22 per cent of all milk customers change dealers within two years. The present figure is estimated at 10–15 per cent for an average for the country. With the usual amount of interest paid to stops probably 5 to 10 per cent are recovered. It is possible with the right handling of complaints and follow up by the right persons to increase this to 37 per cent, and the author states that he has seen figures indicating as high as 46 per cent in his own organization. About 50 per cent of stops are controllable and the other 50 per cent are not. A large per cent of the former should be brought back.
E.F.G.

448. The Practical Application of the Phosphatase Test. D. M. ROGER, Borden's Farm Products, Brooklyn, N. Y. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 9, 233, March, 1939.

The Scharer Rapid Field Test or a slight modification of it was used in the pasteurizing room on 18,000 lots of pasteurized milk. The modification consisted of using amyl alcohol (boiling range 126°–132° C.) for a color solvent instead of normal butyl alcohol. The reason given for this modification was that high colored milk did not give so much yellow color to confuse the reading as when normal butyl alcohol was used. The incubation time was also increased from 10 to 30 minutes to detect more easily slightly underpasteurized samples. The Scharer test is described in detail and an extensive classification of the causes of underpasteurization is given. The author feels that the Scharer Rapid Field Test is reliable and is a great help to the operator when used as a routine test. E.F.G.

449. An Evaluation of the Various Procedures for Making Phosphatase Tests. L. H. Burgwald and E. M. Giberson, Ohio State Univ, Columbus. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 9, 211, March, 1939.

See abstract 431 this issue.

450. The Sanitation of Paper Milk Containers. R. S. Breed, New York Agr. Exper. Sta., Geneva, N. Y. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, Feb., 1939.

An outline map of the United States shows the location of cities where single service milk containers are in commercial use and also where paper mills that make board for these containers are in operation. The majority is in or near New York state. An illustration shows 24 different types of containers which have been used in at least one practical installation. It is stated that about 1,200,000 single service milk bottles are used daily in this country.

The present data refers mainly to the bacteriological problems arising in the making of the containers themselves. Because paper milk containers are so nearly sterile the method used for glass bottles of rinsing with 100 cc. of sterile water is not satisfactory. One method has been to rinse with 10 cc. of sterile water and incubate the entire 10 cc., and another more rigid method has been to add 10 cc. of sterile broth to a container and after incubation record the containers which remain sterile.

Examination of nearly 8000 containers in 1937 and 1938 showed a rather large percentage with no growth at all and 70 per cent with less than 5 colonies per container. The bacteria found were common non-pathogenic, spore-bearing types and some saprophytic micrococci. No pathogenic bacteria survived the temperatures used in preparing the board.

Examination of 824 samples of paper board disintegrated by a special machine showed about 5 per cent to be sterile. Occasional counts of 500 colonies per gram were obtained when mill conditions were not satisfactory. The best coating with paraffin was obtained at 165–175° F. Above 175° F. some board may be exposed and higher counts may be obtained.

Paper milk bottles are capable of meeting very much more severe rinse count standards than the average glass bottle and also more severe standards from the standpoint of pathogenic organisms. E.F.G.

451. The Application of the Resazurin Test in Determining the Quality of Raw Milk and Cream. M. A. Collins, L. M. White, W. H. Chilson, H. G. Turner, and J. R. Rice, United Farmers Cooperative Creamery Association, Boston, Mass. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 5, 104, Feb., 1939.

The results of daily routine tests were studied to determine the advisability of changing from the routine methylene blue reduction test to a routine resazurin reduction test for the control of raw milk and cream supplies. In this work 1 ml. of 0.005 per cent resazurin dye was mixed with 10 ml. of milk or cream. The time required to reduce the resazurin test and the standard methylene blue test to white was approximately equal. Reduction of resazurin to pink in fresh tank car shipments of milk was approximately half the methylene blue to white time. The results of the two tests agree well in selecting high quality milk. When the resazurin test was 3 hours, or over, the bacterial counts usually showed less than 400,000 per ml. and the milk was as good quality as 5.5 hour methylene blue. Resazurin test of 5 to 6 hours have corresponding plate counts under 100,000 per ml. A predominance of the samples of batch raw cream having resazurin pink tests of 2 hours or more have microscopic counts averaging logarithmically 150,000 to 300,000 bacteria per ml. There was an indefinite correlation between the resazurin test and the rise in acidity of fresh pasteurized cream as compared with the same cream raw. The resazurin test can be applied in different laboratories with uniform results when the methods are standardized. E.F.G.

452. Variations in the Fat and Solids-Not-Fat Content of Milk. W. J. Caulfield, C. H. Whitnah, and F. W. Atkeson, Kansas Agr. Exp. Sta., Manhattan, Kan. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, 6, 127, Feb., 1939.

Three-day monthly composites were made upon the milk of 95 individuals in the college herd, representing the Holstein, Ayrshire, Guernsey and Jersey breeds, during the 1936–37 and 1937–38 seasons starting with September. A study was made of (1) the effect of breed, (2) variations within the breed and (3) variations from month to month over a period of one year in the per cent of fat and per cent of solids-not-fat in the composite samples. In the analysis of the 1101 composite samples included in the data two interesting facts were observed. The Holstein breed yielded solids-not-fat averages of 8.52 ± .85 and the solids-not-fat content was below 8.5 for 36 per cent of the animals. The station herd averaged below 8.5 per cent solids-not-fat for 7 out of the 12 months during the period September, 1936, through August, 1937. This would indicate that a solids-not-fat legal standard of 8.5 would exclude much normal milk. Another item of interest was a drop of about 1 per cent in the solids-not-fat content of all milk in the 1936–37 season during July followed by a return to normal in August. The drop in fat content in July was only two-tenths of one per cent. This was a season of drought condition and high temperatures. This drop in solids-not-fat content did not take place during the following summer when adequate pastures and lower temperatures prevailed.

E.F.G.

453. Milk Cartage in the Southwick-Agawam Area of the Springfield Milk Shed. ALFRED A. BROWN AND J. ELIZABETH DONLEY. Mass. Agr. Exp. Sta. Bull. 363, May, 1939.

The bulletin reports only a part of a general study of milk marketing being made of the Springfield milk shed. Factors studied were the supply characteristics of the section, location of farms, markets of wholesale producers, and milk cartage service in the section.

The greatest emphasis is placed on cartage service, going into details on routes operated, efficiency of transportation and trucking rates. A proposed reorganization of service is given as well as a proposed reorganization of rate structure. H.G.L.

454. Feed Flavors and Practical Means of Controlling Them in Dairy Products. C. E. WYLIE AND THOS. B. HARRISON, Univ. of Tenn. Mimeograph Report No. 51.

The methods of controlling flavors in dairy products are divided into three classes as follows: (1) Preventive methods, (2) Processing methods, and (3) Economic encouragement. Each of the subheadings under these is discussed in detail. A table is also included showing a general procedure in controlling flavors. C.E.W.

455. Composition of Milk of Brown Swiss Cows. O. R. OVERMAN, O. F. GARRETT, K. E. WRIGHT, AND F. P. SANMANN. Ill. Agr. Exp. Sta. Bull. *457:* 575–623, July, 1939.

The results of the analyses of 428 milk samples taken from 33 lactations of 17 Brown Swiss cows are tabulated, studied by statistical methods and comparisons made with the results of other investigations into the composition of milk. The samples were 3-day composites taken at 4-week intervals throughout the lactation periods of the individual cows. Summary tables taken from Bull. 325 (1929) of the same experiment station are included. These summaries are based upon the analyses of 208 samples of Ayrshire milk from 22 lactations of 14 cows, 321 samples of Guernsey milk from 33 lactations of 16 cows, 268 samples of Holstein milk from 25 lactations of 19 cows, 199 samples of Jersey milk from 22 lactations of 15 cows and 1,002 samples of the milk of Guernsey-Holstein crossbred cows from 96 lactations of 66 cows. Total number of samples of milk 2,426. It should be pointed out that the protein content of milk as determined from these investigations is higher in relation to the fat content than is usually reported in the literature, and that the lactose does not regularly increase with increasing fat up to a certain fat content but that it is nearly constant in per cent to about 5.0 per cent fat and then decreases with further increases in fat. O.R.O.

MISCELLANEOUS

456. Teaching Dairy Cattle Selection. W. B. Nevens and A. F. Kuhlman. Ill. Agr. Exp. Sta. Circ. 495, June, 1939.

A manual primarily for the use of 4-H Club leaders. The following steps in the process of teaching dairy-cattle selection are considered:

1. Identification and study of the characteristics of the different breeds of dairy cattle.

2. Study and use of the score card for rating dairy cattle.

3. Practice in the comparative selection of dairy cattle on the basis of score-card ratings, and in the preparation of well-organized reasons for the selection.

4. Study of production records and pedigrees—how they are made, and their value and use in the selection of dairy cattle.

5. The use of production records and pedigrees in making selections of animals, and the combining of the use of these records and pedigrees with the selection of animals on the basis of type.

A suggested outline and questions for study are included. O.R.O.

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

457. Rancidity in Edible Fats. C. A. Lea, 1939, Low Temperature Research Station, Cambridge. First American Edition. Published by: Chemical Publishing Company, Inc., New York, N. Y. 230 pages, $4.00.

The author defines rancidity as "a deterioration in odor and flavor which develops in fats or in fatty constituents of food stuffs on keeping," and thus includes in his discussion problems of absorption of odors by such foods as eggs, butter and meat.

The chemistry of fats and the chemical changes produced in them as they become rancid are treated on a broad basis of facts. The outstanding features of the book are: 1) The elucidation of the problem as a whole and the evaluation and interpretation of the work of the various research workers in the field; 2) The inclusion of methods in some detail for measuring the degree of rancidity and interpretations of the meaning and usefulness of the various tests; 3) An extensive bibliography, the chapter on Atmospheric Oxidation, for example, having 324 references; 4) A discussion of procedures to protect foods from rancidity. The book will be of great value to the research worker and all others who deal with the problem of food preservation in its various forms. J.F.L.

458. Butter. Fourth Edition. C. C. Totman, the late G. L. McKay, and C. Larsen. John Wiley & Sons, Inc. 472 pages, illustrated, price $3.50.

This book, formerly entitled "Principles and Practice of Buttermaking" by McKay and Larsen, is designed primarily as a textbook for undergraduate instruction, dairy plant operators and others interested in dairying. The subject matter is presented in twenty-three chapters under the following chapter headings: History of Buttermaking; Composition and Properties of Milk and Cream; Milk and Its Products as Foods; Variation of Fat in Milk and Cream; Care of Milk and Cream on the Farm; Buying and Grading Cream; Neutralization; Pasteurization; Starters and Cream Ripening; Churning, Washing, Salting, and Working of Butter; Overrun; Defects in Butter: Their Causes and Prevention; Butter Marketing; Dairy Equipment: Metals, Depreciation, Care; Refrigeration; Washing Powders; Sanitation; Testing; Starting a Local Creamery; Standards and Definitions; Dairy Production and Statistics; Benefactors of the Dairy Industry, Dairy Associations, and Dairy Journals. The appendix contains a survey of the cream grading laws of the United States, a discussion of butter classification and grades, rules, and regulations governing use of the Iowa butter trademark and the legal requirements of butter.

The buying and grading of cream, churning, working of butter and composition control and marketing are dealt with in considerable detail while related topics are treated in a more general way. Considerable material is presented in graphical form to facilitate instruction.

A list of selected references and questions covering the material presented in each chapter are helpful additions to the text. W.L.S.

459. Standard Chemical and Technical Dictionary. H. BENNETT. Chemical Publishing Co., Inc., New York, N. Y. 638 pages, price $10.00.

This work is a condensed technical word book bearing concise definitions rather than encyclopedic treatments. The coverage of industrial products, chemicals and trade names is complete. Abbreviations and contractions as well as symbols used in chemistry, mathematics, thermodynamics, pharmacy, etc., are given.

A special section on the nomenclature of organic compounds is included. The system of nomenclature followed throughout is based on the International Union Rules. The section on nomenclature is supplemented by the prefix names of the organic radicals.

This technical word book should prove very useful to students, writers, scientists and others who feel the need of keeping up to date with the many new technical words and expressions. T.S.S.

BACTERIOLOGY

460. Methods of Measuring Gas Production. L. A. ALLEN, Agricultural Bacteriology Department, Univ. of Reading, Reading, England. J. Dairy Res., *10:* 1–6, 1939.

An apparatus and methods are described for measuring gas production by microorganisms. Gas volume is calculated from the movement, due to the pressure of the developed gas, of a pellet of mercury in a calibrated capillary tube. Arrangements are described for both aerobic and anaerobic conditions. S.T.C.

461. Protein Metabolism and Acid Production by the Lactic Acid Bacteria in Milk. Influence of Yeast Extract and Chalk. M. BRAZ AND L. A. ALLEN, Agr. Bacteriology Dept., Univ. of Reading, Reading, England. J. Dairy Res., *10:* 20–34, 1939.

Streptococci and lactobacilli were grown in milk and in milk to which yeast extract, chalk or both was added. A study was made of acid production, viability, and effect on the protein in the milk on incubation at

20° C. The growth of streptococci or lactobacilli in milk resulted, with most species, in an increase of protein after several weeks' incubation. The proteose-peptone fraction showed a corresponding decrease. There was considerable proteolysis, however, in the milk used for cultures to which chalk was added. Much more acid was developed by the cultures in milk to which yeast extract was added. There was a decrease in the viability of the cultures (particularly of streptococci) grown in the milk to which yeast extract was added, presumably owing to the lethal effect of the extra acid produced, since when chalk was present the viability was not affected by yeast extract. S.T.C.

462. Gas Production by Bacterium Coli and by Lactic Acid Streptococci under Different Conditions. M. M. HASSOUNA AND L. A. ALLEN, Agr. Bacteriology Dept., Univ. of Reading, Reading, England. J. Dairy Res., *10:* 7–19, 1939.

Using the capillary tube method of measuring gas evolved it was found that with vigorous strains of *Str. lactis* and of *Bact. coli* growing together in separated milk, when the inocula were respectively 1/10 and 1/1000 ml. (of a young milk culture in both cases) gas production by the latter organisms was inhibited by the former. In separated milk the gas evolved during the growth of *Bact. coli* was found to be carbon dioxide only. In dextrose or lactose broths under the same conditions, however, both hydrogen and carbon dioxide were formed. *Str. lactis, Str. cremoris* and *Str. paracitrovorus,* whether in pure or in mixed cultures, gave no evidence of gas formation by the capillary tube method in either separated milk, amounts were detected by the titration method, using large h tubes in which carbon dioxide was absorbed by alkali during growth. S.T.C.

463. The Part Played by Bacteria in the Reduction of Methylene Blue in Milk. BETTY CONSTANCE HOBBS, Dept. of Bacteriology, London School of Hygiene and Tropical Medicine, London, England. J. Dairy Res., *10:* 35–58, 1939.

The author reports data showing that in any given sample of good quality raw milk one type of organism tends to assume predominance at the time of reduction of methylene blue. Members of the coliform group and *Staph. aureus* were among the commonest organisms present at the time of reduction. Various organisms isolated from milk at the time of reduction were examined for their rate of decolorization of methylene blue when growing in pure culture in "sterile" milk (freshly drawn from the healthy udder under aseptic conditions). The most rapidly reducing organisms belonged to the coliform group. Using a differential manometer the same organisms were tested for their power of absorbing oxygen when grown under highly aerobic conditions. With any given organism there was little relationship between the rate of oxygen absorption and the rate of methylene blue reduction. The author's data taken in conjunction with those of other workers shows a fairly high correlation between the rate of fall in the oxidation-reduction potential of the culture and the rapidity with which methylene blue is reduced. The mechanism by which methylene blue is reduced in milk by actively growing bacteria is discussed in some detail. The author believes that reduction is primarily a result of the metabolic reactions proceeding at the cell surface of the bacteria themselves, and consequently that the methylene blue reduction test affords a good index of the extent of bacterial metabolism in the milk. S.T.C.

464. Sur les Alterations Acidogènes du Lait.

(On the Changes Produced in Milk by Acid-Producing Bacteria.) A. FOURNIER. Le Lait, *19:* 575–585, 1939.

An attempt is made to assess the ability of fecal bacteria to ferment the lactose of milk and to compare their rate of acid production with that of true lactic acid organisms. Where milk samples were inoculated and held at 20° and 37° C. both types produced acid with equal speed. The amount of inoculum was without effect. Samples were also held at −15° for 10 hours and at 60, 80, 100 and 105° C. for 30, 20, 15 and 10 minutes respectively, but with no increase in lactic acid occurring. O.R.I.

CHEESE

465. Starter Cultures for Cheese Manufacture. Maintenance of Acid-producing Activity in Cultures of Lactic Streptococci.

H. R. WHITEHEAD AND G. J. E. HUNTER, Dairy Research Institute (N. Z.), Palmerston North, New Zealand. J. Dairy Res., *10:* 120–132, 1939.

Heavy inoculation (1.0–1.6 instead of the usual 0.2 per cent) was found effective in preventing starter failure due to bacteriophage. The authors postulate that the effect of heavy inoculation in eliminating the spontaneous appearance of bacteriophage is due to the more rapid growth of the streptococci when they are transferred to the new batch of milk; *i.e.* to the shortening of the lag period. S.T.C.

466. The White Particles in Mature Cheddar Cheese.

F. H. MCDOWALL AND A. K. R. MCDOWELL, Dairy Research Institute (N. Z.), Palmerston North, New Zealand. J. Dairy Res., *10:* 118–119, 1939.

A sufficient quantity of the white particles for chemical analysis were picked out by hand from a mature cheese. The white specks were shown to be calcium lactate. S.T.C.

CHEMISTRY

467. Séparation du Lactose et des Proteines Solubles Contenues dans le Sérum du Lait par Extraction par L'alcool. (Separation of the Lactose and of the Soluble Proteins of Whey by Extraction with Alcohol.) G. Genin. Le Lait, *19:* 693–698, 1939.

Two variations of an alcohol-extracting method for the separation of lactose from spray-dried whey powder are described. First, the whey powder is mixed with 70.7 per cent alcohol. Water and 95 per cent alcohol are then added at the rate of one part water to four parts alcohol. From the acidified filtrate obtained, a good yield of high quality lactose can be secured.

A more rapid method consists of the use of 95 per cent alcohol at a temperature of 60° C. for two hours. A lower yield of lactose is obtained by this method. The first method described requires an extraction period of 15 hours. O.R.I.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

468. Sanitary Control of Dried Milk. Paul S. Prickett, Mead Johnson and Co., Evansville, Ind. J. of Milk Techn., *2:* 133–137, 1939.

Some of the more important bacteriological procedures which may be applied to the sanitary control of this product are discussed. L.H.B.

469. Laits Dilués, Laits Concentrés, Lait Desséchés. Considérations sur le Problème de la Concentration la plus Convenable du Lait dans les Aliments Lactés Infantile. (Diluted Milks, Concentrated Milks, Dried Milks. Consideration of the Problem of the Most Suitable Concentration of Milk in Infant Milk Foods.) L. Pikler. Le Lait, *19:* 561–573, 1939.

In this review the question of using concentrated milks for infant feeding is studied from several unusual angles such as the effect of changes in the solids concentration upon colloid structure, salt balance, osmotic pressure, protein solubility, etc. No experimental evidence is presented to show that these changes affect the nutritional properties of concentrated milks. O.R.I.

DISEASE

470. Studies in Mastitis. I. The Routine Diagnosis of Mastitis. J. G. Davis, J. McClemont, and H. G. Rogers, National Institute for Research in Dairying, Univ. of Reading, Shinfield, near Reading, England. J. Dairy Res., *10:* 59–73, 1939.

Methods of taking and examining samples of milk for *Str. agalactiae* are described. Where the samples can be examined within a few hours the authors prefer plain blood agar. Edward's medium is recommended where delay in examination is unavoidable. Cultural confirmation of suspected colonies is considered to be imperative. A test for the hydrolysis of hippurate which is said to be the most specific biochemical characteristic of *Str. agalactiae* is described. S.T.C.

471. Studies in Mastitis. II. A Comparison of Methods for the Detection of Subclinical Mastitis. J. G. DAVIS AND J. MCCLEMONT, National Institute for Research in Dairying, Univ. of Reading, Shinfield, near Reading, England. J. Dairy Res., *10:* 74–80, 1939.

Using the results obtained by plating the milk twice in Edward's medium as a criterion, the following were compared:

1. Plating the deposit from 10 ml. of milk in the same medium.
2. Direct plating of milk in Edward's medium without blood.
3. Direct plating of milk in blood agar.
4. Direct plating of milk in milk agar.
5. Methylene blue reduction test.
6. Catalase test.
7. Rennet test.
8. "Growth of starter" test.
9. Brom-cresol-purple paper test.
10. Incubation test (with addition of litmus solution).
11. Titratable acidity test.
12. Clinical examination.

The authors consider the brom-cresol-purple paper, strip cup and induration tests to be worth doing in order to get a general idea of the incidence of mastitis in the herd. All of the simple tests had roughly the same order of efficiency in that they detected about half the positive cases and gave 10–30 per cent false positives. S.T.C.

472. Studies in Mastitis. III. Mastitis in Relation to "Udder" Counts. J. MCCLEMONT AND J. G. DAVIS, National Institute for Research in Dairying, Univ. of Reading, Shinfield, near Reading, England. J. Dairy Res., *10:* 81–87, 1939.

Mastitis was found to have a marked effect on the "udder count" as given by the total colony count on milk agar. Samples for the "udder counts" were taken after about ½ pint of milk had been removed from each quarter. No samples from mastitis-free cows were found to have a count of over 2000 per ml. and very few over 500. About 50 per cent of infected cows yielded milk with a count of over 1000. The authors state that a count of under 100 per ml. may be taken to indicate freedom from infection and a count of over 1000 to indicate mastitis. S.T.C.

473. Studies on Mastitis. IV. Mastitis in Relation to the Methylene Blue Reduction Test. J. McClemont and J. G. Davis, National Institute for Research in Dairying, Univ. of Reading, Shinfield, near Reading, England. J. Dairy Res., *10:* 88–93, 1939.

A systematic investigation of a number of strains of *Str. agalactiae* in milk containing varying concentrations of methylene blue was made. The results confirm previous findings that *Str. agalactiae* either does not reduce methylene blue or does so with difficulty. In testing 156 moderately and severely infected cows only thirty-six gave a short reduction time (9 hr.). Of 252 samples free from infection, twelve gave a false positive reaction. It is concluded that while some cases of mastitis lower the reduction time, the test is of no use for the detection of mastitis. Storage of the samples for 16 hours, either at 4 or 15.5° C., did not materially improve the efficiency of the test. S.T.C.

474. The Value of Indirect Methods for the Detection of Mastitis. S. J. Rowland and M. Zein-el-dine, Dept. of Agricultural Chemistry, Univ. of Reading, Reading, England. J. Dairy Res., *10:* 108–113, 1939.

Using the mean square contingency as a measure of agreement with the direct bacteriological method, indirect methods for the detection of subclinical mastitis were placed in the descending order of their value for diagnosis thus: casein number, chloride content, centrifuge deposit, solids-not-fat content, brom-cresol-purple reaction, brom-thymol-blue reaction, presence of clots, catalase content. S.T.C.

475. Studies in Mastitis. VI. General Observations, Summary and Conclusions. J. G. Davis and J. McClemont, National Institute for Research in Dairying, Univ. of Reading, Shinfield, near Reading, England. J. Dairy Res., *10:* 104–107, 1939.

The incidence of mastitis was found to increase steadily with age of the animal. The authors stress that for eradication purpose there are only two methods of diagnosis worth using—simple tests on the farm and carefully controlled bacteriological examination in the laboratory. S.T.C.

476. Studies in Mastitis. V. Mastitis in Relation to Cheese-making. J. G. Davis and J. McClemont, National Institute for Research in Dairying, Univ. of Reading, Shinfield, near Reading, England. J. Dairy Res., *10:* 94–103, 1939.

A test (brom-cresol-purple-rennet) based on the time of coagulation with rennet, reaction with brom-cresol-purple and type and appearance of curd after incubation for 16 hours at 37° C., is described and recommended as a general test for mastitis and suitability of the milk for cheesemaking. Milk, abnormal due to mastitis, was found to yield cheese of appreciably weaker body (lower viscosity). Many samples of mastitis milk were found to give slow growth of starter. S.T.C.

477. Infectious Bovine Mastitis. 7. Characteristics of Udder Staphylococci. W. N. Plastridge, E. O. Anderson, L. F. Williams and F. J. Weirether. Storrs Agr. Exp. Sta. Bull. 231, 1939.

Staphylococcal mastitis is usually milder in character, and incidence of recovery is greater than with Streptococcal mastitis. The source of *Str. agalactiae* appears to be limited to the infected bovine udder, whereas the source of pathogenic udder staphylococci is apparently not limited to the infected udder.

The following properties did not appear satisfactory for determining the relative irritating ability of a given culture: pigment formation, effect on litmus milk, ability to ferment certain carbohydrates and to liquify gelatin and behavior on alkaline bromthymol-blue lactose agar and Bacto crystal violet agar. However, in general, cultures associated with a high degree of irritation in the bovine udder were more active in laboratory media than were cultures associated with a low degree of irritation.

The ability of recently isolated udder staphylococci to coagulate human plasma was found to be more closely related to irritating ability than any other property.

The average leucocyte count of 2,125 "normal" samples was found to be 73,000 per cc. and the chloride content was 0.121 per cent.

The average leucocyte content of samples of milk containing non-hemolytic, weakly hemolytic and hemolytic staphylococci was 220,000, 1,020,000 and 1,361,000 per cc. respectively. The average chloride content of same samples for nonhemolytic, weakly hemolytic and hemolytic staphylococci was 0.118, 0.141, and 0.151 per cent respectively.

In the routine examination of milk, udder staphylococci which produce hemolytic colonies on cows' blood agar or which are associated with leucocyte counts of 500,000 or more per cc. may be considered as probably pathogenic, providing that high leucocyte counts resulting from early or late lactation are excluded.

The practical consideration of the possibility of food poisoning from milk is discussed. E.O.A.

478. A Further Study of the Production of Milk of Abnormal Composition by Cows Free from Udder Streptococci. E. G. Hastings and B. A. Beach, Wis. Agr. Exp. Sta. J. Agr. Res., *58:* 7, 543–556, April 1, 1939.

The foremilks of 12 of 31 cows previously studied throughout their first lactations (J. Agr. Res. 54: 199–220, 1937) were examined periodically during their second lactations for chlorides and catalase, using the first 30 cc. from each quarter as sample. At less frequent intervals the remainder of the milk from each quarter was examined in greater detail. Disturbances noted in the foremilk during the first lactation were observed also during the second lactation. This continued study did not reveal the cause of the disturbance or the transmissibility of its cause. Abnormal milks produced without apparent bacterial cause were noted and the authors believe that the environment provided in the abnormal quarters is more favorable for the establishment of streptococci than that provided by the normal gland. Milk from disturbed quarters was rarely so changed in composition as to be of less value either as fluid milk or as a raw material for milk products than normal milk. L.M.T.

479. Report of the Committee on Communicable Diseases Affecting Man. I. A. Merchant, Iowa State College, Ames, Iowa. J. of Milk Techn., *2:* 110–117, 1939.

There were 43 milk-borne epidemics reported in the United States and 13 reported in Canada during 1937.

The origin of 18 of the epidemics in the United States was traced to carriers. Raw sweet milk was involved in the majority of cases. Where pasteurized milk was involved, investigation showed that the process was faulty or the milk was contaminated after pasteurization.

A number of epidemics, all of which were gastrointestinal in nature were traced to milk products (cheese and homemade ice cream).

From 1923 through 1937 there have been a total of 653 milk-borne epidemics reported in the United States.

In order to inform milk handlers of effective methods in preventing diseases being spread by milk, the Connecticut State Department of Health has published a pamphlet which has been distributed to dairymen. A copy of this pamphlet is made a part of this report. L.H.B.

480. The Relation of Streptococcic Mastitis to Certain Phases of Milk Sanitation. C. S. Bryan, Mich. Agr. Exp. Sta., East Lansing, Mich. J. of Milk Techn., *2:* 32–40, 1939.

Physical examination of the udder is of great value in detecting streptococcic mastitis, but is not as reliable as the microscopic test.

The microscopic test is based upon the continued presence of streptococci of more than six organisms per chain in milk of infected cows. The milk must be properly collected and incubated at 37° C. for at least 12 hours before examining under the microscope.

This test was more efficient than seven other tests commonly used for detecting mastitis.

The city of Lansing, Michigan, requires Grade A raw milk to be produced from cows that are free of tuberculosis, Bang's disease, and streptococcic mastitis. L.H.B.

FEEDS AND FEEDING

481. Feeding Grass Silage to the Dairy Cow.

H. S. WRIGHT, Sheffield Farms Company, Pawling, N. Y. Cert. Milk, *14:* 155, 8, 1939.

The author concludes that the molasses ensilage method of crop preservation merits a part in the Sheffield Farms feeding plan when applied:

1. Through the medium of lower protein roughages fed to the dry cow and young stock.

2. Through the higher protein, better milk making roughages in the ration of the milking cow, in combination with hay and concentrates in amounts necessary to furnish the food requirements. W.S.M

FOOD VALUE OF DAIRY PRODUCTS

482. The Effect of Churning on the Vitamin A and Carotene Content of Milk Fat.

K. M. HENRY AND S. K. KON, National Institute for Research in Dairying, Univ. of Reading, Shinfield, near Reading, England; A. E. GILLAM, Chemistry Dept., Victoria Univ., Manchester, England, AND P. WHITE, Univ. of Reading, Reading, England. J. Dairy Res., *10:* 114–117, 1939.

The vitamin A and carotene content of 14 samples of butterfat secured by churning and by ether extraction from the same milks were determined by spectrophotometric methods and by Lovibond tintometer tests. Assuming that there is no loss of either carotene or vitamin A in the course of the ether extraction, their data indicates that there is some loss of carotene in churning but that vitamin A is not appreciably affected. S.T.C.

483. De la Vitamine A et du Carotene dans la Conservation du Lait et la Creme sous Pression D'oxygène Élevée (Procédé Hofius).

(Studies on the Vitamin A and Carotene in Milk and Cream Preserved by Increased Oxygen Pressure, (Hofius Process.).) E. BROUWER, E. A. M. MEYKNECHT AND N. D. DIJKSTRA. Le Lait, *19:* 673–686, 1939.

Samples of milk and cream held for one to three months under about ten atmospheres pressure and at 4–6° C. showed no significant loss of vitamin A or Carotene. Analyses were made by spectrophotometer and in five of the nine experiments by rat assays. Both raw and pasteurized products were studied. In one experiment the temperature of holding was raised to 19° C. without increasing the rate of destruction of vitamin A or its precursor. O.R.I.

484. Le Taux de L'acide Ascorbique dans de Lait de Quelques Mammifères. (The Ascorbic Acid Content of the Milk of Some Mammals.) L. BURIANA, Le Lait, *19:* 449–455, 1939.

Recent literature is reviewed to show that there is some disagreement regarding the ascorbic acid content of the milk of different species and especially during the secretion of colostrum. As a result of recent studies the authors contend that the method of Martini and Bonsignore (Biochem. Z. *273*: 170, 1934) based on the reduction of methylene blue is a more accurate quantitative method than the one utilizing 2-6-dichlorophenol-indophenol.

Human milk, analyzed by this method was shown to be very poor in ascorbic acid. Values obtained from several analyses averaged around 3 mgm. per liter whereas the same samples analyzed by the 2-6-dichlorophenol-indophenol method gave values varying between 30–50 mgm. per liter. The values found for mares', asses', and sows' milks were all extremely high. Cows' milks varied between 50–70 mgm. per liter when analyzed by the methylene blue method. O.R.I.

485. Influence des Manipulation et de la Température de Conservation sur le Contenu en Vitamine C du Lait Destiné a L'alimentation Humaine. (The Effect of Handling and of Holding Temperature on the Vitamin C Content of Milk Destined for Human Consumption.) G. FAILLA, Le Lait., *19:* 455–461, 1939.

Using Sharp's method of determining vitamin C with 2-6-dichlorophenol-indophenol, the author found most samples of commercial pasteurized milks were much lower in this vitamin than was the raw milk of the University of Milan herd. A study of the factors suspected of causing this difference revealed that:

(1) Vitamin C is oxidized to marked degree in raw milk. This is particularly true where the raw milk is not held at a low temperature.

(2) Oxidation takes place at a greatly reduced rate in pasteurized milk.

It is suggested that pasteurization destroys the peroxidase enzyme which is the agent causing rapid oxidation of the vitamin. O.R.I.

486. Factors Affecting the Vitamin C Content of Milk. MYER GLICKSTEIN AND J. H. FRANDSEN, Mass. State College, Amherst, Mass. Milk Dealer, *28:* 11, 42–46, Aug., 1939.

A review of the literature dealing with effect of stage of lactation, breed, ration, miscellaneous factors, and commercial practices on the vitamin C content of milk. C.J.B.

HERD MANAGEMENT

487. Lower Production Costs a Vital Factor. H. B. ELLENBERGER, Univ. of Vermont, Burlington, Vt. Assoc. Bull., Intern. Assoc. Milk Dealers, *10:* 1, 22, Dec. 20, 1938.

Rather than to depend upon higher prices of milk to furnish a profit it would produce satisfactory results more surely to apply known methods of reducing the cost of producing 100 pounds of milk on the farm by employing already known selection breeding and feeding methods. At least 7,000 to 8,000 pounds of average test milk per cow should be sought. Generous use of good quality grass and hay provide much cheaper feed costs per 100 pounds than too heavy grain feeding. It is stated that the feed cost of producing milk with poor roughage is 25 per cent to 30 per cent higher than with good roughage. E.F.G.

488. Problems in Growing Herd Replacements. G. E. TAYLOR, New Jersey Agr. Exp. Sta., New Brunswick, N. J. Part I, Cert. Milk, *14:* 158, 7, 1939; Part II, Cert. Milk, *14:* 159, 11, 1939.

Herd health and disease control is one of the major problems confronting the owner of livestock. As a disease preventive measure it is suggested that dairy farmers adopt a practice of growing heifers that are needed for replacement purposes. The problems encountered in growing herd replacements are discussed, beginning with a calf at time of birth and following through with recommended practices leading up to the time the heifer comes into production. W.S.M.

ICE CREAM

489. Condensed and Evaporated milk with Reference to Ice Cream. P. A. DOWNS, Univ. of Nebr., Lincoln, Nebr. J. of Milk Techn., *2:* 130–132, 1939.

Evaporated milk, which is sterilized, presents no bacteriological problem to the ice cream manufacturer if used as soon as the cans are opened.

Sweetened condensed, either skim or whole if it contains 72 to 74 per cent total solids, presents no problem either. However, when modifications are used so that there isn't sufficient sugar present to make a saturated solution of the water left in the product then the product must be so handled to prevent bacterial growth. Usually this product is frozen solid and stored at such temperature to prevent thawing until ready for use.

Plain condensed and super-heated condensed milk must also be handled in this way.

Care must be exercised in thawing these products before use so as to prevent contamination and increase in bacterial numbers. L.H.B.

490. Public Health and Aspects of Fruits, Nuts, Colors, and Extracts in Frozen Desserts. M. J. PRUCHA, Univ. of Ill., Urbana, Ill. J. of Milk Techn., *2:* 127–129, 1939.

Bacteriological examinations were made of a considerable number (207) of samples of ingredients used in ice cream, such as flavoring extracts, fruits, nuts, and color solutions. Results showed that 164 samples contained bacteria of various kinds, 24 samples had an abundance of coliform bacteria present, and 107 samples contained yeasts and molds.

A study of the bacteriological condition of these various ingredients made at two different plants, one where the ingredients were properly cared for and the other where no care was given, showed that proper care of the ingredients was a big factor in maintaining not only a lower total bacterial content, but no yeasts and molds were present, and very few coliform organisms. L.H.B.

491. Sanitation of Products Added to Frozen Desserts. P. H. TRACY, Univ. of Ill., Urbana, Ill. J. of Milk Techn., *2:* 118–126, 1939.

More attention should be given by the dairy plant operator in preparing and caring for flavoring and coloring materials used in ice cream. By so doing the sanitary qualities of the ice cream will be improved.

Methods of preparation and precautions to be used for fruits, nut meats, coloring material and flavoring extracts are given.

Certain fruits and extracts will withstand heating to 145° F. for 30 minutes without serious injury to the flavor.

Nut meats should be dipped in boiling sugar solution (50 per cent), to which one per cent salt has been added, for 15 seconds, and then dried in an oven for 2.5 minutes at 250° C. L.H.B.

492. Sediment Tests on Frozen Desserts. M. E. PARKER, Beatrice Creamery Co., Chicago, Ill. J. of Milk Techn., *2:* 87–90, 1939.

It is suggested that the sediment test on ice cream be made on a half pint sample of mix or a pint sample of the frozen ice cream.

The same sediment standards which are used for milk may be applied to frozen desserts.

References as to methods for determining the amount of sediment in various products used in making ice cream mix are given. L.H.B.

493. Notes on Microbiological Analysis of Ice Cream. A. H. ROBERTSON, State Food Laboratory, Albany, N. Y. J. of Milk Techn., *2:* 84–86, 1939.

Methods of taking samples and making bacterial counts on ice cream are discussed. L.H.B.

494. What Are Frozen Desserts? F. W. FABIAN, Mich. State College, East Lansing, Mich. J. of Milk Techn., *2:* 75–83, 1939

General definitions of various frozen desserts are given. Inconsistencies of legal definitions are cited. There is a wide variation in the range of minimum legal requirements of chemical composition of frozen desserts.

Sanitary requirements have been slow in coming but are on their way. In 1926 no state had a bacterial standard while now 12 states have a minimum bacterial standard for ice cream ranging from 75,000 to 500,000 per gram.

A standard of not more than 100,000 bacteria per gram should be established as should a pasteurizing temperature of 150° F. for 30 minutes.

The problem of what to do about frosted malted milk is discussed but no solution is given. L.H.B.

495. Weight and Overrun. M. C. VARNEY, Engineer, Exact Weight Scale Co., Columbus, Ohio. Ice Cream Field, *34:* 2, 42, Aug., 1939.

A description of a scale suitable for determining overrun in ice cream and ices is described, and the relation between overrun and density illustrated. W.C.C.

496. Refrigerated Truck Bodies. H. H. HARRINGTON AND L. M. S. COOPER, Transportation Dept., General Electric Co., Erie, Pa. Ice Cream Field, *34:* 2, 12–14, 43–47, Aug., 1939.

This is a fairly complete report with recommendations for design and construction of refrigerated truck bodies suitable for ice cream. The various phases are well illustrated with pictures or schematic diagrams.

According to the report there is a demand for the following type of refrigeration equipment.

1. Automatic. Direct expansion evaporator with condensing unit having electric motor for plug-in power and power take-off drive. For service requiring unlimited operating time and with the truck engine running equivalent to at least 10 miles per hour of elapsed time.

2. Automatic with partial hold-over including condensing unit, a-c motor for plug-in power and PTO drive. For service requiring unlimited operating time and with the truck engine running equivalent to an average of 6 miles per hour of elapsed time. This system is especially well suited to mixed interurban and city service.

3. Hold-over with condensing unit and motor for plug-in power. For frequent stop, city service and low daily mileage. The time away from the plant is limited by the thermal capacity of the hold-over cooling units.

4. Hold-over for plant refrigeration. This system is preferred by operators using a large number of trucks operating from a central plant in which a large ammonia refrigeration system is available. For service similar to No. 3, except that facilities for recharging are limited to the ammonia plant.

The normal insulation thickness recommended is 6 inches except on the roof crown where it is 8 inches. On this basis and with the design of equipment proposed the insulation occupies approximately 40 per cent of the total body volume leaving 60 per cent for useful load.

With this relatively large insulation volume it is advisable to select insulation with low specific weight. Using glass wool and thermocraft the total body insulation equals 400 pounds, if cork were used the insulation weight would be approximately 1400 pounds.

Although color and sign painting are generally specified by the operator it is advisable to use light colors and it is especially desirable to have the roof painted aluminum as a means of decreasing solar heat absorption.

It is pointed out that to obtain the maximum effectiveness of the hold-over cooling unit, especially in extremely warm weather, it is advisable to scrape the bottom surface of the tank daily, particularly when the humidity is high.

Methods of arriving at required compressor capacities as well as costs of operation under various conditions are illustrated by calculated example.

W.C.C.

MILK

497. Factors Influencing the Reduction of Methylene Blue in Milk.

C. K. Johns, Central Exp. Farms, Ottawa, Can. Sc. Agr., *21:* 435–457, 1939.

This paper is a comprehensive report on the effect of variations in methods upon methylene blue reduction time. An incubation temperature of 37.5° C. was found to be just as satisfactory as any other. Inversion of the tubes at two hour intervals increased the reliability of the test. More frequent inversion was not advantageous. Increasing the concentration of the dye to 1:300,000 resulted in a 20 per cent longer reduction time.

A preliminary incubation period of 18 hours at 12.8° C. was shown to improve the test as an index of keeping quality. Fresh milk possesses a bacteriostatic principle which inhibits the multiplication of bacteria and reduction of the dye. The use of the incubation period at 12.8° C. lessens the effect of this principle and permits closer grading of the milk. O.R.I.

498. Factors Affecting the Composition of Milk.

D. H. Jacobsen and G. C. Wallis. South Dakota Bull. 331, 1939.

Analyses of milk from cows of the College herd were made monthly over a 5-year period. The milk from Holstein, Guernsey, Jersey and Ayrshire cows was analyzed for fat, total solids, ash, calcium and phosphorus. The influence of breed, stage of lactation, and season was studied.

Fat tests in general decreased to the end of the third month and then increased with the advance in lactation. Jersey and Guernsey milk tested highest near the end of the lactation; Holstein milk tested highest at the beginning of the lactation.

Total solids analyses showed Holstein milk tested highest at the beginning of the lactation.

Total solids analyses showed Holstein milk to average 10 to 11 per cent, Guernsey about 14 per cent and Jersey 14 to 15 per cent.

Holstein milk showed greater tendency to be sub-legal in solids-not-fat than in fat; the former being below standard throughout the year.

Calcium and phosphorus decreased the first month and increased during the last 3 months of lactation.

Colostrum milk was also analyzed. It is suggested that low testing milk can be avoided by: (1) selecting cows for high test (2) balancing freshening dates during the year (3) standardizing low-test with high-test milk.

C.C.T.

499. Why Sales Contests? R. G. Peat, Silverwood Dairies, Ltd., Hamilton, Ont. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st Year, *4:* 89, February 13, 1939.

A great value of sales contests is that they break the regular routine and lift salesmen out of a rut. The spirit of competition properly developed by suitable incentives, the strongest of which are frequently not money, will improve the salesman's approach to the customer. Sales contests return a profit to the company. E.F.G.

500. What Consumers Think of the Milk Dealer. Paul Potter, Associated Milk Dealers, Inc., Chicago, Ill. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st Year, *4:* 84, February 13, 1939.

The Business Research Corporation of Chicago carefully sampled the Chicago milk market through 1038 personal interviews with housewives and 350 interviews with retail grocers.

Some of the conclusion are:

1. Losses in volume by the dealers are due more to depression, reduced prices on other foods and cut-rate competition than to dissatisfaction with the product or to any change in attitude toward the use of milk or to the dealers selling it.

2. The public wants both the home wagon delivery service and the neighborhood store distribution.

3. That the storekeepers are satisfied to handle milk as a convenience on a minimum profit margin and that this is a highly desirable situation for the public and for the milk dealer.

4. The belief that selling through stores increases the per capita consumption is based upon experiences in which the price differential between the wagon price and the store price was a great deal more than 1 cent per quart.

5. The situation calls for careful examination of possible ways of reducing wastes in production processing and distribution methods and the

possibility of wage adjustments based upon what the public will pay as well as what the wage earner wants.

6. There is a need for a better understanding of the food value of milk, and a need for more and better reminders as to how and when to use it.

7. The value of the good will built up over the years by good products and good services warrants extraordinary efforts to preserve it. E.F.G.

501. Guide Posts for Hiring Route Salesmen. MARLYN A. SMULL, Univ. of Tenn., Knoxville, Tenn. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st Year, *1:* 77, February 13, 1939.

Since the cost of training route salesmen is considerable there is need for a sound method of selecting the most promising men for this work.

Thirty-two definable personal differences among milk salesmen were classified by 16 milk sales executives from standpoint of their relative importance for sales success. Adaptability was considered the most desirable quality. Fifty route salesmen were rated on desirable characteristics by means of a rating scale and their score compared with their sales record. The validity of the characteristics for sales work was demonstrated. The order of importance of the characteristics is as follows: 1. Adaptability; 2. Alertness; 3. Business-likeness; 4. Originality; 5. Initiative; 6. Drive; 7. Judgment; 8. Self confidence; 9. Tact; 10. Patience.

The author suggests a questionnaire which could be answered in 30 minutes which would enable the employer to rate salesmen on the above characteristics. E.F.G.

502. A Study of Pasteurized Milk in Rochester, N. Y., Employing the Phosphatase Test. HAROLD W. LEAHY, Health Bureau Laboratories, Rochester, N. Y. J. of Milk Techn., *2:* 147–151, 1939.

The phosphatase test used consisted of adding 1.0 cc. of milk to 10.0 cc. of Kay and Graham's buffered substrate solution and incubating for 18 hours at 37° C. Then 0.2 cc. of a 0.4 per cent solution of BQC was added to determine the presence of phenol.

Three standards were used for determining the degree of pasteurization. These were made by adding 0.01 mg., 0.05 mg. and 0.10 mg. of phenol, respectively, to pasteurized milk.

Milk producing 0.01 mg. or less of phenol was interpreted as "properly pasteurized"; between 0.01 and 0.05 mg. as "slightly underpasteurized," and between 0.05 mg. and 0.10 mg. as "underpasteurizerd." Those which produced greater amounts than that were definitely "underpasteurized."

Using this method in the course of a year's study of 104 pasteurizing plants in Rochester, New York, 50 plants were observed whose milk never gave evidence of improper pasteurization. Twenty-one plants were found with occasional minor imperfections, and routine phosphatase tests were rarely positive. The remainder frequently showed evidence of faulty operation; nine of these on inspection showed multiple faults. L.H.B.

503. Latest Developments in Cooling Milk on the Dairy Farm. John E. Nicholas, Pennsylvania State College, State College, Pa. J. of Milk Techn., *2:* 141–146, 1939.

Four types of modern mechanical milk coolers are illustrated and described. They are either of the type spraying cold water around the neck of the can or the can is submerged to the neck in cold water. In all cases the water is agitated during the cooling process.

When the initial temperature of the cooling water was 36.5° F. and the initial temperature of the milk was 90° F. and there was 3.5 gallons of water for each gallon of milk cooled, the temperature of the milk at the top of the can at the end of one hour was 53° F. and at the bottom of the can was 46.5° F. The temperature of the water at the end of 1¼ hours when agitation was stopped was 46.5° F. L.H.B.

504. Report of the Committee on Dairy Farm Methods, 1938. F. D. Holford, Borden Farm Products Co., New York City. J. of Milk Techn., *2:* 138–140, 1939.

This report deals almost entirely with the cleaning and care of milking machines.

It is not necessary to take machines apart after each milking. Taking apart once a week to prevent the rubber tubing from sticking to the metal parts is sufficient. Check valves, vacuum lines, etc. should be cleaned regularly.

Two methods are recommended, No. 1–Cold and hot water (180° F.) are drawn through by suction immediately after milking. Then protect the units by storing in a dry place or in clean cold water, brine or chlorine solution until the next milking. Use a solution.rack.

No. 2–Cold water and lye solution. Cold water is drawn through the units with vacuum; the long milk tube and teat cups are then hung on a solution rack and filled with a weak solution of lye. (This method should not be used on milking machines having aluminum parts as lye corrodes aluminum.) L.H.B.

505. Reduction of Milk Losses in Milk Plants. A. C. Dahlberg, J. C. Hening, and H. L. Durham, New York Agr. Exp. Sta., Geneva, N. Y. J. of Milk Techn., *2:* 96–99, 1939.

See abstract 520 this issue.

506. Engineering Problems in Milk Sanitation. Leslie C. Frank, U. S. Public Health Service, Washington, D. C. J. of Milk Techn., *2:* 58–72, 1939.

Milk sanitation is a problem which requires the serious attention of sanitary engineers as well as of bacteriologists, veterinarians, and epidemiologists.

In addition to the problems of pasteurization the sanitary engineer should give attention to the following problems:

1. Dairy barn and milk house design.
2. Dairy farm water supplies.
3. Disposal of excreta on the farm.
4. Pasteurization plant design.
5. Water supplies.
6. Plumbing.
7. Excreta and waste disposal.
8. Special problems relating to pasteurization equipment, operation and design to insure every particle of milk being properly pasteurized. Regenerators, leak protector valves, air space heaters, automatic controls, etc.

L.H.B.

507. The Practical Application of the Phosphatase Test. D. M. Roger, The Borden Farm Products Co., Brooklyn, N. Y. J. of Milk Techn., *2:* 21–25, 1939.

Scharer's rapid field test or slight modification of it was found reliable when properly used. More than 18,000 tests were made during the past year in the Borden plants.

No cases of interfering substances in milk have been found.

Modifications generally used were to substitute amyl alcohol in place of butyl, and to increase the incubation time to 30 min. in place of 10 min.

Amyl alcohol has the advantage of not extracting so much of the yellow color from high color milk and cream.

Increasing the incubation time made the test more sensitive.

Some causes of high phosphatase tests found are given. L.H.B.

508. Homogenized Milk. C. J. Babcock, Bureau of Dairy Ind., Dept. of Agr., Washington, D. C. J. of Milk Techn., *2:* 26–31, 1939.

Homogenized milk is on the increase. According to a questionnaire sent out by the Bureau of Dairy Industry to milk plants all over the United States, 260 replies have been received. Of these 30 reported that they were featuring homogenized milk, and 23 of them reported that the sale of homogenized milk was increasing.

Homogenized milk, pasteurized at 143° F. for 30 minutes, at 2500 lbs. pressure the average curd tension was reduced to 16.7 gr. as compared to an average curd tension of 50.2 gr. for the raw milk.

Digestion studies made of raw milk, milk boiled for five minutes, and pasteurized milk, homogenized at 2500 lbs. pressure, showed that the boiled and homogenized milk digested more rapidly but not any more completely than the raw milk. The boiled milk digested slightly more rapidly than did the homogenized. These tests were made in vitro. L.H.B.

509. Application of the Rapid Phosphatase Test to Dairy Products. HARRY SCHARER, Chemical Laboratory, Dept. of Health, New York City. J. of Milk Techn., *2:* 16–20, 1939.

Phenol standards previously prescribed for the Scharer laboratory test have been replaced by a set of more stable inorganic standards. Instructions for the preparation of these standards are given.

The laboratory test may be increased in its sensitivity by increasing the incubation temperature above 100° F. to 115° F. or by using a two hour incubation period in place of one hour.

Both Scharer's laboratory test or his rapid field test may be applied to milk, cream, chocolate drink, butter, cheese, and ice cream. Instructions for application of the test to these various products are given.

Wherever interfering substances are present, which may give a false positive test, they may be determined by repeating the test substituting buffered water in place of the buffered substrate.

Certain precautions are given. L.H.B.

510. The Pouring Lip and Cap as Sanitary Factors in Bottled Milk. LLOYD ARNOLD, M.D., Dept. of Bacteriology and Public Health, Univ. of Ill., College of Medicine, Chicago, Ill. J. of Milk Techn., *2:* 41–45, 1939.

The pouring lip is a source of contamination, and the development of a glass milk bottle which reduced the area to be covered to a minimum and without drip on pouring is important.

This area should be covered with a sanitary closure to safeguard the bottle content. The plug or disc cap alone, in general use today, does not properly protect the contents. L.H.B.

511. The Present Status of the Paper Milk Container. F. W. TANNER, Dept. of Bacteriology, Univ. of Ill., Urbana, Ill. J. of Milk Techn., *2:* 4–15, 1939.

The paper milk container has certain advantages over glass, particularly from the standpoint of public health. That paper containers when properly made, are almost free of bacteria is proven by examination of thousands of containers of one type. Very few viable bacteria of any kind and no pathogens nor *Escherichia coli* were found. L.H.B.

512. Product-Costs of Milk to Dealers in the Springfield Area, 1935. ALFRED A. BROWN AND J. ELIZABETH DONLEY. Mass. Agr. Exp. Sta. Bull. No. 365, July, 1939.

The factors considered and analyzed in the study are significant of average price, 1935 price level, product-costs to dealers, month to month variation in individual dealer's product-costs, causes of variations in dealers costs F.O.B. the market, cause of variation in percentage of Class II handled by dealers, characteristics of the industry that hinder rapid adjustment in production, alternatives to a market pool, and the relationship between the price for Class I and Class II usage. H.G.L.

513. Draw a Picture for Your Producers. JOSEPH BURNS, Schwartz Milk Co., Two Rivers, Wis. Milk Dealer, *28:* 10, 94, July, 1939.

The author points out the advantages of using the microscope and the sediment test with producers as a means of bringing about the production of good-quality milk. C.J.B.

514. Ropy Chocolate Milk. C. A. TARNUTZER, Bacteriologist, Robert A. Johnston Company, Milwaukee, Wis. Milk Dealer, *28:* 10, 42, 84, July, 1939.

A brief discussion is given of the causes of ropy chocolate milk. C.J.B.

515. Reducing Butterfat Loss.. WILLIAM S. JERMAIN, Sparks Dairy, Inc., Buffalo, N. Y. Milk Dealer, *28:* 10, 41, July, 1939.

The installation of a motor-driven agitator in the weight can resulted in a large saving in butterfat loss. Prior to installation of the agitator the average test of composite samples of the milk received from the patrons was about 0.2 per cent higher than the test of the milk processed and the drip test of the milk received. C.J.B.

516. Treatment of Paper Milk Bottles to Delay or Prevent the Oxidized Flavor of Milk. C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Pennsylvania State College. Milk Dealer, *28:* 11, 29, 66–69, Aug., 1939.

Oxidized flavor was delayed or prevented when the milk was stored in paper bottles which had been coated with a mixture of paraffin and oat flour (Avenex). The milk, however, frequently had a flour flavor, the bottles softened and bulged, and the paraffin frequently floated on the milk.

Evidence is presented to show that paper milk bottles made from board which has been sized with oat flour before paraffining aid in preventing or delaying the development of an oxidized flavor in milk. C.J.B.

517. Churned Buttermilk. ANONYMOUS. Milk Dealer, *28:* 11, 32, 83, Aug., 1939.

The Carlson-Frink Company, Denver, Colorado, has opened up an entirely new class of business by selling churned buttermilk. The buttermilk is made by churning sweet cream and sells for 2 cents more per quart than cultured buttermilk. The new product has not adversely affected the sale of cultured buttermilk. C.J.B.

518. Cutting Milk Delivery Costs. ANONYMOUS. Milk Dealer, *28:* 11, 30–31, 50–54, Aug., 1939.

The cost of operating old trucks *versus* new trucks is shown by comparative data covering 75 delivery trucks operated by milk dealers for home-to-home delivery. The total operating cost for the old trucks was .2232 cent per mile and for the new trucks .1781 cent per mile. This represents a saving of .0451 cent per mile or $45.10 for each 1,000 miles. C.J.B.

519. The Value of the International Association of Milk Dealers from an Educational Point of View. W. H. E. REID, Univ. of Missouri, Columbia. Assoc. Bull., Intern. Assoc. Milk Dealers, *10:* 1, 28, Dec. 20, 1938.

This Association has functioned not only as an aggressive trade organization but as an effective educational institution because of the care with which its technical sectional programs have been built up and conducted. E.F.G.

520. Reduction of Milk Losses in Milk Plants. A. C. DAHLBERG, J. C. HENING AND H. L. DURHAM, New York, Agr. Exp. Sta., Geneva. Assoc. Bull., Intern. Assoc. Milk Dealers, *10:* 1, 11, Dec. 20, 1938.

The daily losses in eight plants in New York State were determined. In one plant it was found that with 3.9 per cent fat in the original milk there was 6.0 per cent fat in the can drainage while the vat rinsings corrected for dilution contained 14.4 per cent fat. Skimming all drainage and rinsings in one plant receiving 96,000 pounds of milk per day resulted in the recovery of 69 pounds of fat in the cream which yielded butter scoring 93. One plant receiving 28,000 pounds of 3.8 per cent milk per day and in which equipment was simple and losses kept at a supposed minimum showed a loss of 3 pounds of fat per day.

The average receipts at the 8 plants was 62,442 pounds of milk containing 2,148 pounds of fat from which 2.5 pounds of fat was lost daily which should have gone into the dump vat. The total losses of fat per plant were 13.6 pounds per day although a portion of this was actually being recovered in various ways.

It is suggested that a supporting bar over the weight can would permit a can to drain while the next one is being dumped and would not materially slow down the receiving process. Or a drainage position ahead of the cold water rinse might be used. Prevention of avoidable losses would also usually solve the stream pollution problem. In this study, extent of dilution of milk in rinsing was measured by a freezing point determination. E.F.G.

521. Grading of Market Milk. B—Reporting Grading Results to the Producer. R. C. DAYTON, Rieck-McJunkin Dairy Co., Pittsburgh, Pa. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st Year, *2:* 53, Jan. 31, 1939.

Among several methods of reporting unsatisfactory quality of milk to the producer a tag in two parts with a check list of the more common defects is recommended. The first part of the tag is tied to the can and the second part kept for future reference. Abnormal conditions should be reported promptly and if possible followed up with a personal letter. E.F.G.

522. Grading of Market Milk. A—Producer's Quality Records. FRANK J. CAPOUCH, Bowman Dairy Co., Chicago, Ill. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st Year, *2:* 51, Jan. 31, 1939.

The importance of fat, lactometer, sediment and bacterial tests as well as flavor and odor in grading of market milk is discussed. E.F.G.

523. Milk Production Trends. JOHN L. WILSON, Bureau of Agr. Ec., U. S. D. A., Washington, D. C. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st Year, *2:* 35, Jan. 31, 1939.

The milk cow is a flexible unit and her production may be increased or decreased as conditions dictate. Total milk production is regulated by two major factors, first, the number of milk cows, and, second, the rate of milk production per cow. During the seven months prior to this time (fall, 1938) normal numbers of dairy cows in relation to population and generous feed supplies have favored heavy production. Heavy feeding will likely continue during the winter. Eighteen cows per 100 are usually discarded per year and replaced by 18 heifers if the number of milk cows is to remain constant. Through decreased culling and increased retention of heifers the stage is set for an increase in numbers of cows beginning one or two years hence in about 1940. This perspective increase might however be interrupted by drought, unfavorable prices or other factors which cannot be accurately forecasted. Also since the cow is a flexible unit her output will be regulated somewhat by the price of the product. E.F.G.

524. Single Service Containers for Milk and Milk Products. J. R. SANBORN AND R. S. BREED, New York State Agr. Exp. Sta., Geneva, N. Y. Cert. Milk, *14:* 154, 7, 1939.

A general discussion of methods for bacteriological control in the manufacture of milk container board is given. The sanitary condition of the foundation board greatly influences the sterility of the paper bottle. A tentative board count of 500 colonies per gram has been set as a reasonable and enforceable standard.

The authors conclude that when food container board is made from virgin pulp, using pure process water and under sanitary methods of manufacture, a product is produced which is entirely suitable for direct contact with dairy products. W.S.M.

525. Critical Discussion of Some Methods and Standards for Certified Milk.

J. H. Brown, Johns Hopkins Univ., Baltimore, Md. Cert. Milk, *14:* 154, 5, 1939.

Reasons for regarding the supervision of certified milk production as a special problem requiring special methods and standards are given. Special points discussed are: (1) certain features of medical milk commission control, (2) the bacteriological methods and standards for certified milk, and (3) pasteurization of certified milk. W.S.M.

526. An Economic Study of Milk Distribution in Maine Markets.

George F. Dow, Assoc. Economist, Maine Agr. Exp. Sta. Bull. 395, March, 1939.

A detailed analysis of the costs of milk distribution in representative Maine cities and towns obtained by the survey method from 266 milk distributors of which 89 per cent were of the producer-distributor type. It was found that plant costs averaged 36 per cent, container costs 5 per cent, selling and delivery costs 51 per cent, and administration costs 8 per cent. The cost of distribution for the largest raw milk distributors was approximately one cent per quart less than for those with the smallest volume. The pasteurized milk distributors had higher costs than the raw milk distributors, partly because of pasteurization costs and partly because of other costs, such as higher wages. The delivery cost for man and delivery equipment was approximately the same with horses as with motor vehicles for routes up to 15 miles in length. L.M.D.

MISCELLANEOUS

527. Bilateral Variations in the Weight and Composition of the Long Bones of Some Experimental Animals.

Chas. E. Weakley and R. B. Dustman, W. Va. Agr. Exp. Sta., Morgantown, W. Va. J. Agr. Res., *58:* 9, 711–716, May 1, 1939.

Left and right side bones of rats, chickens, swine and dairy cattle were compared as to green weight and content of moisture, ether extract, ash and organic non-fat. All the animals had been on feeding trials designed to produce differences in bone development and bones of both control and experimental animals were studied. Some of the samples were composites of related bones, *i.e.,* femur, tibia and fibula was one of the composites, seventh and eighth ribs another, etc. No significant differences were found in weight of green bone or in moisture, ash, and organic non-fat of the rat.

Green bone weight was higher for the left side bones of chickens and swine and for the right side bones of dairy heifers than for bones of the opposite sides. Whenever the bones of one side were significantly higher in green weight and moisture than those of the other side they were lower in percentage of ash. The authors conclude that certain species of lower animals may tend toward a dominance of one side over the other with respect to bone development, and that bones from one side are not necessarily identical in composition or other properties with those of the other side, although in general the differences found in their experiments were quite small.
L.M.T.

528. Wage Scales and Working Conditions in Dairy Plants. ANONYMOUS. Milk Dealer, *28:* 11, 41, 61–64, Aug., 1939.

Results of a survey conducted by the Milk Dealer showing methods of payment, especially commission payment plans, methods used in splitting routes, vacations, work-day schedules, etc., in dairies in various parts of the country and including those in small towns. C.J.B.

529. Suggestions for Calculating Cooling Loads for Dairies. C. T. BAKER, Consulting Engineer, Atlanta, Ga. Milk Dealer, *28:* 11, 35, 82, Aug., 1939.

A brief discussion is given of the properties of milk which influence its specific heat and its freezing point. The author also outlines the information which is necessary for calculating the amount of heat to be taken up by the refrigerating machine for any given case. C.J.B.

530. Need for Proper Training of Our Salesmen. T. KLINE HAMILTON, Diamond Milk Products, Inc., Columbus, Ohio. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st Year, 4, 71, Feb. 13, 1939.

This article is a discussion of the use and effectiveness of the I. A. M. D. Balanced Job Program in training route-salesmen. E.F.G.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

531. A Microbiological Assay for Riboflavin. E. E. SNELL AND F. M. STRONG, Univ. of Wis., Madison, Wis. Ind. Eng. Chem., Analyt. Ed., *11:* 6, 346–50. 1939.

A biological method is described for estimating the riboflavin in natural materials by determining the amount of growth of *Lactobacillus casei* in a medium containing the unknown sample. To a riboflavin-free basal medium is added a prepared extract of the unknown, the mixture is inoculated and incubated and the amount of growth determined by measurement of acidity or turbidity of the culture. The reliability of the method is supported by agreement of the assay results at different levels, recovery of added riboflavin, successful determination of riboflavin in the presence of photolyzed extracts, and specificity of structure required for activity. A series of assays on the same product at two-week intervals yielded results which compared well.

B.H.W.

532. Influence of Bacteria on Oxidation of Ascorbic Acid. WILLIAM B. ESSELEN, JR., Dept. of Bacteriology and Physiology, Mass. State College, Amherst, Mass. Food Research, *4:* 4, 329. 1939.

As a result of studies with forty-five different strains of bacteria (39 species), it was concluded that most of the organisms significantly retarded oxidation of ascorbic acid, especially when they were cultured in media containing readily fermentable carbohydrates. No evidence was found that bacteria are destructive to ascorbic acid, nor did any of the organisms studied appear to synthesize the vitamin. F.J.D.

BUTTER

533. Factors Affecting Amount of Mold Mycelia in Butter. J. ADAMS AND E. H. PARFITT, Purdue Univ., Lafayette, Ind. Nat'l Butter and Cheese J., *30:* 10, 29. 1939.

When initial contamination is held constant, mold mycelia fragments in butter are an index of the length of time, temperature of storage, and the oxygen relationship of the cream from which the butter was made. Manufacturing operations do not influence the mold mycelia content of butter.

W.V.P.

534. Composition Control with Special Reference to the Rolless Churn. C. C. TOTMAN, S. Dakota State College, Brookings, S. D. Nat. Butter and Cheese J., *30:* 8, 12. 1939.

The roll-less churn produced butter of remarkably uniform composition.

W.V.P.

CHEESE

535. A Study of Winter and Summer Made Cheddar Cheese. J. C. MARQUARDT, N. Y. Agric. Exp. Sta., Geneva, N. Y. Nat. Butter and Cheese J., *30:* 9, 28. 1939.

Cheese made from winter milk is "low quality" because it lacks flavor when cured at low temperatures. Ten lots of winter-made raw-milk cheese and 9 lots of summer cheese from New York state factories were scored, analyzed and given a cheese flavor index value during ripening; four lots of winter-made Canadian cheese and 7 lots of Wisconsin summer cheese were given the same inspection. All were cured at 35 to 40° F. Like studies were conducted using cheese made experimentally from high-quality, raw and pasteurized milk. Some of the experimental cheeses were cured at 35 to 40° F., others at 55° F. after holding 2 weeks at 35 to 40° F., the remainder were cured at 55° F. The factory cheese made in summer developed greater flavor intensity in a shorter time than did the cheese made in winter; the quality of the two types as indicated by the average scores was quite similar. The experimental cheese made in summer showed the same seasonal effects. Curing at low temperatures delayed good as well as bad flavor development but produced cheese of better quality. Curing raw-milk cheese first at high then low temperature lowers the flavor and body score and produces off flavor development which makes it difficult to evaluate flavor intensity. Combinations of low and high temperature curing are suggested to force cure. W.V.P.

CHEMISTRY

536. The Concentration of Ionized Magnesium and Calcium in Milk. R. NORDBÖ, Dept. of Physiology, Univ. of Oslo, Oslo, Norway. J. Biol. Chem., *128:* 745. 1939.

The results of analysis of Na, K, Ca, Mg, Cl, inorganic phosphate, and citric acid and of the determination of H and Mg indicate a value of the ionic strength of about 0.075 to 0.08 in ultra filtrate of fresh market milk. The K of calcium citrate was found to be 3.16×10^{-4} and of magnesium citrate 2.65×10^{-4}, at 37° and ionic strength of 0.08. Lactose combines with calcium and magnesium to form non-ionized compounds. The concentration of magnesium ions in ultra filtrates of fresh market milk is 0.4 to 0.5 mM. per liter. Total diffusible Mg is about 2.5 mM. per liter, of the diffusible Ca of fresh market milk (8.5 to 10 mM. per liter) only about 20 per cent is present as calcium ions. K.G.W.

537. Kniaseff Fat Test. HORACE H. SELBY AND THELMA A. SELBY, Hage's, Ltd., San Diego, Calif. Ind. Eng. Chem., Analyt. Ed., *11:* 7, 393–94. 1939.

The fat test of Kniaseff was found to compare with the Mojonnier fat test on ice cream and ice milks more favorable than any of 25 other modifications of the Babcock method which were investigated by the authors. The method is essentially one of alkali-alcohol-ether extraction carried out in the conventional Babcock cream test bottle by the addition to the sample of two reagents made up according to the directions given. The loaded bottles are centrifuged, the fat column read and a correction applied which gives results generally within 0.1 per cent and always within 0.4 per cent of values obtained by the Mojonnier method. B.H.W.

538. Xanthine Oxidase: Purification and Properties. Eric G. Ball, Kaiser Wilhelm-Institut für Zellphysiologie, Berlin-Dahlem, Germany. J. Biol. Chem., *128:* 51. 1939.

A process is described whereby an enzyme preparation is obtained from whole milk, and which is 500 times more active per unit of dry weight than the original material. Evidence is presented that this enzyme is a flavoprotein. K.G.W.

539. Determination of Riboflavin in Milk by Photoelectric Fluorescence Measurements. David B. Hand, Cornell Univ., Ithaca, N. Y. Ind. Eng. Chem., Analyt. Ed., *11:* 6, 306–09. 1939.

A fluorometric method for determining the riboflavin in milk or whey which is rapid, accurate, and specific is described. Fifty ml. of acetone is added to 25 ml. of milk and the fluorescence of the filtrate is compared with that of a cube of uranium glass. The fluorescence intensities of samples of riboflavin were determined and the uranium glass was calibrated against solutions containing known amounts of the pigment. The apparatus developed for making the fluorescence measurements consisted of a photoelectric cell, a microameter, a capillary mercury lamp, two glass filters, a block of uranium glass and a square glass bottle. B.H.W.

DISEASE

540. Relationship between Carotene, Blindness Due to Constriction of the Optic Nerve, Papillary Edema and Nyctalopia in Calves. L. A. Moore. J. Nutrit., *17:* 443–459. 1939.

For the past 20 years various investigators have noted a peculiar type of blindness in calves associated with rations where poor quality or no roughage was fed. Previous work has shown that the blindness is due to a constriction of the optic nerve where it passes through the optic foramen caused by a stenosis of the bony canal. Further work with calves has shown that the blindness is preceded by nyctalopia, and ophthalmoscopic observations revealed papillary edema and bleaching of the Tapetum Lucidum. Holstein calves placed on the blindness producing ration and fed crystalline carotene dissolved in cottonseed oil in quantities sufficient to keep the blood plasma carotene level well above a lower limit of 0.13 micrograms per ml. did not develop these eye changes. Vitamin A deficiency is therefore the causative factor.

In mature cows nyctalopia and papillary edema develop but blindness due to constriction of the optic nerve does not develop due to the fact that the bony optic canal has already been formed. In calves blindness due to constriction of the optic nerve and papillary edema are associated, while nyctalopia is a separate process but due to the same deficiency.

Papillary edema is a cardinal sign of increased intracranial pressure. Vitamin A may therefore be necessary for the regulation of this pressure. Increased intracranial pressure may also account for certain nervous manifestations of vitamin A deficiency. C.F.H.

FEEDS AND FEEDING

541. Carotene Content of Different Varieties of Green and Mature Soybeans and Cow Peas.

W. S. Sherman and W. D. Salmon, Labratory of Animal Nutrition, Ala. Polytechnic Inst., Auburn, Ala. Food Research, *4:* 4, 371. 1939.

Most varieties of soybeans in the green stage where found to be very good sources of carotene ranging from 212 to 705 γ per 100 grams of fresh sample. The varieties of cow peas studied contained from 140 to 231 γ per 100 grams of fresh sample. The carotene content of mature soybeans ranged from 8 to 243 γ per 100 grams of air-dry sample. All varieties of mature cow peas were low in carotene, being comparable to the lowest soybean varieties. Reduction of as much as 50 per cent in carotene resulted from allowing plants to stand in the field two or three weeks after the seeds had reached maturity.

F.J.D.

542. Beobachtungen über den Einfluss verschieden grosser Heugaben auf Kraftfutterverbrauch und Gedeihen der Kälber. (Observations on the Influence of Varying Amounts of Hay on Consumption of Concentrates and Growth of Calves.)

L. Paloheimo, Institut für Haustierlehre der Universität Helsinki. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *9:* 4–6, 287–293. 1937.

A continuation of work reported in earlier numbers of this Journal (9, 52 and 234).

When skim milk (plus cod liver oil) was fed alone, the amount given at individual feedings was of significance in the growth of the calves, for when the portions were small the ill effect of lack of roughage became apparent in the second month, whereas when large individual portions of skim milk were fed this effect was not evident until the fourth month.

If no hay was fed, larger quantities of ground oats were eaten, but the quantity of corn meal eaten was unchanged. The calves suffered as much when fed skim milk and corn meal as when fed skim milk alone.

When no hay was fed and ground oats was the concentrate the calves throve nearly as well as when both hay and ground oats were fed. Ground oats seems to be a natural mixture of roughage and concentrate.

With limited hay feeding the calves' requirements for concentrates were the same with either corn or oats. If hay was offered in abundance the consumption of ground oats was limited more sharply than that of corn meal. If all constituents of the ration were offered *ad lib*, consumption of hay was about the same regardless of whether the concentrate was corn meal or ground oats. J.G.A.

543. Die wissenschaftliche Grundlage des stärkwertes. (The Scientific Basis of Starch Values.) J. Axelsson, Institut für Haustierfütterung der Landw. Hochschule Schwedens, Uppsala. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *11:* 3, 176–205. (2 Abbildungen.)

A review and restatement of the problem in the light of recent additions to knowledge of the subject. Optimal contents of crude fiber in the dry matter and of protein in the digestible organic substance are necessary if metabolizable energy available for gains in live weight is to be utilized as completely as possible. Eighteen to 23 per cent of crude fiber and 10 to 18 per cent of protein are considered optimal. Therefore, when the effect of a ration is to be determined by difference, both the basal ration and the feed (or ration) on trial must contain optimal quantities of fiber and protein. As this was not the case in Kellner's work his starch values are open to serious criticism. Information concerning the fiber and protein content of rations regardless of the feeds which compose the rations is, however, not sufficient. Care must be taken that the content of other substances with special nutritive values is optimal. Because of this, relative values of feedstuffs cannot be determined on a net calorie basis but instead must be judged by the energy content at the beginning of the production process. Content of metabolizable energy seems to deserve preference over content of digestible energy. J.G.A.

544. Die stimulierende Wirkung des Proteins im Tierfutter. (The Stimulating Effect of Protein in Feeds.) J. Axelsson, Institut für Haustierfütterung der Landw. Hochschule Schwedens, Uppsala. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *11:* 3, 162–175. (3 Abbildungen.)

A review of the more recent work on the subject. The author summaries as follows:

Gains in weight increase and simultaneously feed energy is better utilized when the protein content of the feed is raised to a certain degree (not stated). A further increase in protein causes a decrease in gains and also in energy utilization. The optimal protein content for gains in weight diminishes with the growth of the animal. Utilization of the protein for deposit begins to decrease long before the optimum for gains in weight is reached, and continues to decrease after this point is surpassed. Simultaneously a definite and continuously increasing part of the nitrogen and energy of the feed is being excreted in the urine. Thus the favorable effect of the protein, when offered in optimal quantity, is manifest, even though only a reduced amount of it is stored in the body. Therefore a stimulating effect must be assumed.

During lactation also there seems to be an increased utilization of feed energy when protein is increased, and that beyond the quantity of protein needed for nitrogen balance in the body. The optimal quantity seems to be higher than hitherto has been supposed. Whether the protein content necessary for highest milk yield corresponds to that for best energy utilization is still in doubt. As a result of this change in viewpoint on effect of protein, the deciding factor in formulating protein standards for practical use must be the utilization of the total ration rather than that of the portion used only for production. J.G.A.

545. Über einen Einsäuerungsversuch mit Zusatz von Ammonbicarbonat und Fütterungs versuch mit dem erhaltenen Gärfutter an Milchkühen. (Experiments on the Making and Feeding to Milch Cows of Ensilage with Added Ammonium Bicarbonate.) C. WINDHEUSER, Landesversuchsanstalt für landw. Chemie, Landw. Hochschule Hohenheim, Biedermann's Zentralblatt, Abteilung B., Tierernährung, *11:* 2, 139–146. 1939.

German yellow maize was fermented in three silos, NH_4HCO_3 being added as small crystals to the contents of one silo at chopping time. All the silages were of good quality, and were fed to milking cows to ascertain whether the nitrogen of the ammonium salts formed in the process (chiefly lactate and acetate) could replace a portion of the food protein.

Effect on milk yield was noted of substituting 51.3 grams of N from ammonia in 15 kilos of silage for 47.8 grams of N from the digestible protein in 1.2 kilo of rape cake. No effect of the nitrogen from ammonia could be noticed; in one period when silage without ammonium salts was fed, and rape cake was omitted, milk yield was not significantly lower than when silage with ammonium salts was fed. J.G.A.

546. Zur Beifütterung von gedämpften Kartoffeln an Saugkälber. (Feeding Steamed Potatoes to Young Calves.) H. STEINHARDT, Agrikulturchemischen und Bakteriologischen Institut der Schleisischen Friederich-Wilhelms-Universität. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *11:* 2, 66–120. 1939. (3 Abbildungen.)

Ten calves fed whole milk, steamed potatoes and malt were compared with six calves fed whole milk alone. Metabolism balance trails beginning after one week on the ration and continuing for four weeks were conducted with seven of the group receiving potatoes and with four of the controls.

Digestive troubles were experienced with four of the ten calves fed potatoes; two of them recovered. Cases of diarrhea were not considered to be caused by the potatoes.

Data are given for digestibility and starch values of both rations. The potatoes had a distinct protein sparing effect, and the animals made greater gains in live weight than those on milk alone. Detailed directions are given for preparing and feeding the potato-malt-milk gruel. J.G.A.

547. Untersuchungen über den Wert von Harnstoff, Glykokoll, Amidflocken und-schnitzeln für Milchkuhe und wachsende Schafe. (Studies of the Value of Urea, Glycocoll, Amide Flakes (Potato) and Amide Cuttings (beet) for Milch Cows and Growing Sheep.) W. Kirsch und F. Sauer, Tierzucht-Institut der Universität Konigsberg. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *10:* 6, 451–480. 1938. (1 Abbildung.)

Several digestion trials were conducted with cows and one with sheep; some of the trials were prolonged and nitrogen balances were ascertained in every case.

If urea and glycocoll are fed in the pure form they must be introduced into the ration very gradually and carefully, otherwise health disturbances and marked reduction of milk yield will result.

When urea was fed in combination with potato flakes or beet cuttings, the mixture was readily eaten in quantities of 1.2–1.5 kilos and proved entirely beneficial.

Urea nitrogen was completely assimilated by both cows and sheep regardless of whether the urea was fed as such or in combination with the amide flakes or cuttings.

In the trials with cows, when the urea-amide-flake or cutting mixture was substituted for ground soybeans so that the urea nitrogen amounted to 20 per cent of the total nitrogen intake, the urea nitrogen was found to have a value for productive metabolism equivalent to from 47–66 per cent of the value of the nitrogen in the basal ration. J.G.A.

548. Über die Einwirkung der Eiweissunternährung auf die Milchproduktion frischmelkender Kühe. (The Effect of Limited Protein Feeding on the Milk Production of Fresh Cows.) P. Kajanoja, Landwirtschaftliche Institut Mustiala in Finnland. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *9:* 4–6, 525–530. 1937.

Cows that had recently calved and were fed considerably less protein than is called for by generally accepted feeding standards, in most cases showed no decrease in milk yield as compared with their records in former years. Nor did an increase in the protein of the ration have any effect in raising their production. Only when the amount of protein supplied was so small that the cows received only as much or about as much true protein as they were secreting in their milk, was a decrease in milk production noted. In such cases an increase in the protein was followed by an increase in milk yield.

These facts indicate that the original ability of wild cattle to take protein from their body substance (chiefly from their muscles) to sustain milk production, still exists in the highly bred races of present day dairy cows to an extent scarcely to be expected.

A very small supply of protein seemed to effect a decrease in fat content of the milk, and conversely, increasing the protein in the ration resulted in increased fat production. Thus it is seen that fat content is influenced more than quantity of milk by a reduction in the protein allowance.

J.G.A.

549. Über die Ensilierung unter Zusatz von Molkereiabfall-produkten und über die Korrelation zeischen pH, Ammoniakzahl, Buttersauregehalt und Geruch bei Grassilagen. (Ensiling with Additions of Dairy By-Products and Correlation between pH, Ammonia Number, Butyric Acid Content and Odor in Grass Silages.) E. BROUWER, Physiologischen Abteilung der Rijkslandbouwproefstation in Hoorn, Niederlande. Biedermann's Zentralblatt, Abteilung B., Tierernährung *9:* 4–6, 508–524. 1937. (9 Abbildungen.)

One hundred twenty-one samples of silage (86 of them from grass) which had been ensiled with whey and other dairy by-products, were examined.

Most of the samples were of inferior quality. Evidently protein-rich crops and Netherland young grass are too high in protein and too low in sugar to permit satisfactory preservation by addition of whey, etc.

Frequency curves of the pH values, the butyric acid contents, and the ammonia fractions (ammonia N as per cent of total N), appeared to be polymodal, even if only one crop (grass) was considered. The polymodality is the expression of different bacteriological processes.

On the average the pH values decreased as the samples contained less protein in the dry matter and less moisture. One per cent of moisture more or less corresponded in grass silage with 0.03 pH units; one per cent of crude protein (ammonia included as 6.25 × N) in the dry matter more or less with 0.07 pH units.

The ammonia content increased considerably with rising pH. At pH 4.0 in grass silage an average of 8 per cent of the total nitrogen was present as NH_3, at pH 5.0 it reached 24 per cent and at higher pH values correspondingly higher NH_3 values were observed.

Butyric acid in the grass silage was very low at pH 4.0; above this value the pH and the butyric acid rose simultaneously and by about the same number of units.

In grass silage especially the odor gives a rather good idea of the pH range and the bacteriological type. In judging the quality of silage for practical purposes, in many instances a simple examination of the odor and the pH is sufficient, although it is not to be implied from this that more detailed investigation is valueless. J.G.A.

550. Der Wert der Amide bei der Fütterung des Rindes. Historische Betrachtung der Entwicklung der Amidfrage, kritische Wertung des Standes unserer heutigen Kenntnisse. (The Value of Amides in the Feeding of Cattle. An Historical Review of the Development of the Amide Problem, and a Critical Evaluation of the Status of our Present Knowledge.) K. Krebs, Institut für Tierzuchtung und Haustiergenetik der Universität Berlin. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *9:* 4–6, 394–507. 1937.

A comprehensive review with a bibliography of 126 references. From a summary of all the evidence the author concludes that the system of rationing farm animals based on digestible crude protein is unsound, since the function of the non-protein nitrogen fraction is not comparable to that of the protein moiety. Although non-protein nitrogen must be taken into account, it must be clearly distinguished from protein nitrogen. J.G.A.

551. Über die Bestimmung des verdaulichen Rohproteins auf Künstlichen Wege. (The Determination of Digestible Crude Protein by Artificial Means.) P. Schwarze, Kaiser-Wilhelm-Institut für Züchtungsforschung Müncheberg/Mark. Biedermann's Zentralblatt, Abteilung B., Tierernährung, *9:* 4–6, 346–354. 1937.

A report of trials to ascertain the digestibility of crude protein in fodder plants, especially legumes. Digestion was effected with pepsin-hydrochloric acid and pancreatin-soda. Trichloracetic acid was used to precipitate the indigestible nitrogen compounds. In several cases control experiments were run with rats.

Hydrolysis and digestion in the organism is incomplete; the greater part of the excrement nitrogen is precipitated by trichloracetic acid and is in turn largely hydrolyzed by proteolytic enzymes.

Under the conditions of the experiment it has been possible to classify nitrogen compounds into those which are hydrolyzed by proteolytic enzymes and those which distinctly are not.

In leguminous seeds and rye digestibility was complete or nearly so, while in alfalfa plants, and corn and barley (grain) under the same conditions a residue was left which was not precipitable by trichloracetic acid. The nature of this residue could not be determined; it may or may not be protein. J.G.A.

552. Determination of Carotene in Silage. An Improved Method. D. M. HEGSTED, J. W. PORTER AND W. H. PETERSON, Univ. of Wis., Madison, Wis., Ind. Eng. Chem., Analyt. Ed., *11:* 5, 256–58. 1939.

An improvement in the accuracy of the carotene determination of silage whereby pigments other than carotene are removed from the silage extracts by the use of a diacetone solution is reported. The presence of foreign pigments in the carotene solution has heretofore often caused the determinations by the old Willstätter-Stoll method and its subsequent modifications to be too high. The authors have substituted a solution of 100 volumes of diacetone and 6 volumes of water for the usual 90 per cent methyl or 85 per cent ethyl alcohol solution used to remove foreign pigments from the carotene solution. Comparative data are given for the alcohol and acetone methods. The solutions from both methods were subjected to chromatographic analysis. B.H.W.

FOOD VALUE OF DAIRY PRODUCTS

553. The Influence of Different Levels of Milk Intake on the Acid-Base Mineral Balances of Children. H. A. HUNSCHER, F. C. HUMMEL AND I. G. MACY. J. Nutrit., *17:* 461–471. 1939.

The nitrogen, acid base and excess base balances are given for seven healthy children, ages 5 to 10 years, when they were consuming 400, 600, 800, and 1,000 gm. of milk daily. All the children were storing both acid and base throughout the entire study but greater quantities were stored with larger intakes of milk. C.F.H.

554. The Utilization of the Calcium of Milk by Pre-School Children. G. KINSMAN, D. SHELDON, E. JENSON, M. BERNDS, J. OUTHOUSE AND H. H. MITCHELL. J. Nutrit., *17:* 429–441. 1939.

Data were presented from a study of the calcium metabolism of five pre-school girls and six pre-school boys. The calcium level in the diet was increased by increasing the milk solids in the diet. The mean value for the calcium utilization of the eleven subjects was 19 per cent. The limited ability of the pre-school child to utilize calcium is entirely different from results with rats. C.F.H.

555. The Utilization of the Calcium in Various Greens. MARY SPEIRS, J. Nutrit., *17:* 557–564. 1939.

The calcium in turnip greens was about as available as that in milk, while that in tender green collards and kale was slightly less available. Not only was the calcium in New Zealand spinach utilized poorly, if at all, but also the utilization of the calcium of the milk in the diet was diminished by the presence of the New Zealand spinach. C.F.H.

ICE CREAM

556. Whipping Capacity of Ice Cream Mixes. ALAN LEIGHTON AND ABRAHAM LEVITON, Bu. Dairy Ind. U. S. D. A., Washington, D. C. Ind. Eng. Chem., *31:* 779. June, 1939.

The effect of variations in manufacturing procedure and mix ingredients upon the whipping properties of ice cream mix is graphically presented. The whipping capacity of each mix is shown by an overrun line in which the highest overrun obtainable at a given temperature is plotted against that temperature. The resulting straight line is an equilibrium line which is characteristic of every mix of a given composition.

The overrun lines of many mixes are presented. They show that the aging of a mix in which butterfat and improvers are absent will increase its shipping properties if the mix has been heated; that an increase in homogenization pressure increases stability and maxium overrun while an increase in homogenization temperature under some conditions accelerates aging. Butterfat is an overrun depressant, especially when the fat is derived from butter, but if the butter is homogenized with skim milk and the reconstituted cream again homogenized with the mix, whipping properties are greatly improved. There is some tendency of sugar to raise overrun but this is offset by its detrimental effect upon stability. Gelatin and carob bean extract have similar effects on the overrun line. Sodium alginate acts as a dual stabilizer, stabilizing the structure and the fat emulsion.

Studies on the temperature-overrun relationship indicate proper adjustment of the freezing process, will lower freezing time, and clarify existing ideas concerning the whipping properties of mixes. B.H.W.

557. A Comparison of Gelatin and Sodium Alginate as Stabilizers in Ice Cream. P. H. TRACY AND S. L. TUCKEY, Dept. of Dairy Husbandry, Univ. of Ill., Urbana, Ill. Food Research, *4:* 4, 335. 1939.

When sodium alginate and gelatin were used in optimum amounts in ice cream there was no decided preference for either stabilizer as far as the body of the ice cream or the whipping time was concerned. No differences in flavor were detectable and no off-flavors attributable to the stabilizers were encountered. Melt-down trials showed ice cream containing sodium alginate to melt more rapidly and to have a smoother appearance than that containing gelatin. F.J.D.

MILK

558. Irradiated Milk. Vitamin D Potency as a Function of Energy Input. BRIAN O'BRIEN, H. DOUGLAS MCEWEN AND KENNETH MORGAREIDGE. Ind. Eng. Chem., *30:* 7, 839–842. 1938.

The results of a study on the antirachitic activation of whole milk by direct action of ultra-violet energy from carbon arcs is presented. A theory is developed which gives a means of predicting the amount of energy input to the arc necessary to produce a given vitamin D potency in the treated milk. As the energy input increases the vitamin content of the milk approaches a maximum value. With continued irradiation the peak in antirachitic potency is passed and a gradual decline occurs with slow decomposition of the active principle. B.H.W.

559. Cereal Flours as Antioxidants in Dairy Products. W. S. MUELLER AND M. J. MACK, Dept. of Dairy Industry, Mass. State College, Amherst, Mass. Food Research, *4:* 4, 401. 1939.

Studies revealed that oat flour, whole oat flour, and corn flour have similar antioxidative properties when used in milk. Wheat (blended and whole) barley, rye and rill flours possess little antioxidative value. In ice cream, corn flour gives similar antioxidative protection to that given by oat flour and has more effect than the latter as a stabilizer. F.J.D.

560. The Irradiation of Milk. K. G. WECKEL AND H. C. JACKSON, Univ. of Wis., Madison, Wis. Wis. Agr. Exper. Sta. Res. Bull. 136. March, 1939.

Irradiation of milk is a process of exposing milk under proper conditions to radiant energy to increase its antirachitic value (vitamin D potency). Milk is a good carrier of vitamin D because it contains a desirable balance of the antirachitic minerals, calcium and phosphorous. Milk has vitamin D as a natural constituent which varies with several factors such as individual cows, feed, and exposure of cows to sunlight. Radiant energy from sunlight has wave lengths which can change certain substances to vitamin D. The chief activatable substance in milk is believed to be 7-dehydrocholesterol. Artificial sources of radiant energy to do this work are carbon arc lamps, quartz mercury vapor lamps, and cold quartz lamp tubes. Radiation from these sources differs quantitatively and qualitatively. Types of equipment designed to use these sources are illustrated and described. Methods of controlling the process of irradiating such as milk flow and radiation intensity are discussed. Factors affecting efficiency of irradiation are inter-related. Those mentioned are milk composition, concentration of activatable substances, opacity of milk; thickness, velocity and turbulence of milk film; intensity and composition of radiation; and mechanical factors affecting characteristics, capacity, and dimensions of the film as well as distance from the source of and angle of incidence of radiation.

Commercial irradiation does not destroy vitamins A, B or G, is only indirectly responsible for slight, variable reduction of C, and reduces slightly the bacterial content. Milk unduly exposed to ultra-violet light may develop a typical "activated" flavor which is associated with the protein fractions of milk. Radiation with other than the antirachitic wave lengths, though not as effective, may produce this flavor. Normal irradiation procedures cause no detectable changes in composition or digestibility of milk. This article is 55 pages in length, contains 54 figures, and 85 references.

W.V.P.

PHYSIOLOGY

561. Influence of the Hypophysis on Carbohydrate Metabolism.

SAMUEL SOSKIN, H. LEVINE AND W. LEHMANN, Dept. of Metabolism and Endocrinology, Michael Reece Hospital, and the Dept. of Physiology, Univ. of Chicago. Am. J. Physiol., *127:* 3, 463. 1939.

An anterior pituitary extract (Phyone) which was shown to be capable of maintaining the carbohydrate levels of fasting hypophysectomized animals, was studied for its effect on the utilization of carbohydrate.

This hormone maintains blood sugar level by stimulating the liver to gluconeogenesis rather than by acting upon the extra-hepatic tissues of the body. The reduction of hyperglycemia, glycosuria and ketosis in the depancreatized animal following hypophysectomy, is due to a decrease in the rate of hepatic gluconeogenesis, and not to a greater utilization of the sugar by the extra-hepatic tissues. D.L.E.

562. Significance of Amino Acids for the Maintenance of Nitrogen in the Adult White Rat.

PAUL A. WOLF AND RALPH C. CORLEY, Laboratory of Biochemistry, Purdue Univ., Lafayette, Ind. Am. J. Physiol., *127:* 3, 589. 1939.

Previous work has shown that the nitrogen of mixtures of amino acids would spare completely that of the tissues of rats, fed diets low in protein. The present work indicates that adult female white rats also require for maintenance of nitrogen balance each of the amino acids essential for growth. Rations practically devoid of protein but supplemented with vitamin preparations and mixtures of the nine amino acids essential for growth, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine, have proven satisfactory for maintaining nitrogen equilibrium in the growing as well as in the adult animal. D.L.E.

563. Further Investigations Concerning the Effects of Formalin on the Gonadotropic and Thyroid-Stimulating Hormones of Cattle Anterior Pituitary Glands in the Guinea Pig.

LEO LOEB AND S. G. HAYWARD, Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine, St. Louis, Mo. Am. J. Physiol., *127:* 3, 497. 1939.

Immersion of single cattle pituitary glands in formalin solutions causes a marked reduction or complete suppression of the leuteinizing and atresin effects and in many instances permits a full maturation of follicles in the ovary of the injected guinea pig. The largest number of positive results were obtained if heifer pituitaries were immersed in 0.25 per cent solutions of formalin at pH 5 for seven days.

It is possible to maintain cattle pituitary glands, which have been converted by the action of formalin into maturation-producing and estrogenic-agents in the sex organs of the guinea pig, unchanged for at least four months, but presumably for even longer periods by immersion in glycerin.

The thyroid hormone is injured or destroyed by formalin. This action is independent of the leuteinizing or atresin effects which are produced in the ovaries. This confirms the conclusion that leuteinizing, atresin and thyroid-stimulating actions are not due to the same agent. D.L.E.

564. The Role of Sex, Estrogenic Hormone, Fasting and Diuresis in the Response of Crop Sacs of Pigeons to Prolactin.

ROBERT W. BATES, OSCAR RIDDLE AND ERNEST L. LAHR, Carnegie Institution of Washington, Sta. for Exper. Evolution, Cold Spring Harbor, N. Y. Am. J. Physiol., *127:* 3, 422. 1939.

When prolactin is injected subcutaneously into immature pigeons, the crop-sacs of males usually give a greater (growth) response, but following intramuscular injections of prolactin there is little or no sex difference in response.

The apparent antagonistic action of estrogen against prolactin is found thus to be largely though probably not wholly the result of the diuresis and reduced food intake which dihydroestrone induces in pigeons; any additional or residual effect is thought likewise to rest not upon a specific antagonism but upon one or another of the general effects of dihydroestrone on metabolism.

Estrogen injected simultaneously with prolactin into pigeons inhibits the gains in body weight which, like the response of growth in the crop sacs, characterize the normal action of prolactin in pigeons; and this inhibition of gain in body weight is apparently produced in the same indirect ways as is the inhibition of the crop-sac response. D.L.E.

565. The Role of the Pituitary in the Calorigenic Action of Vitamin D.

A. BARTOLI, J. FELDMAN AND C. I. REED, Dept. of Physiology, Univ. of Ill. College of Medicine. Am. J. Physiol., *127:* 3, 552. 1939.

The calorigenic response of normal dogs to doses of vitamin D do not occur following hypophysectomy. Both the pituitary and the thyroid are necessary for the calorigenic response to vitamin D. The calorigenic response is largely independent of the effect of vitamin D on mineral metabolism. D.L.E.

566. The Partition of the Serum Globulins of the Dairy Goat. E. P. Reineke, V. E. Peterson and C. W. Turner, Dept. of Dairy Husbandry, Univ. of Mo., Columbia, Mo. J. Biol. Chem., *128:* 1. 1939.

A study of the (sodium sulfate) fractionation pattern of the serum globulin complex of the goat revealed the following fractions: euglobulin, pseudoglobulin I, and pseudoglobulin II. Subdivision products of the latter two groups were obtained. K.G.W.

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